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Manuscripts and editorial correspondence should be sent to the Editor-in-Chief. At least 1 of the authors must be a member of the American Phytopathological Society. Type on only 1 side of sheet; double-space all parts of manuscript, including footnotes, citations, and legends. For papers longer than 1 printed page, the article should begin with a brief, concise summary. Number footnotes consecutively with arabic numerals, except in tables where lower case letters are used. Give each table a heading stating its contents. Make references to literature as footnotes only when 5 or fewer citations are given. Name and address of the institution where the work was done should precede "Literature Cited." List references alphabetically in "Literature Cited" and number consecutively throughout text as well as in list of citations. Until further notice, authors are advised to read "The preparation of manuscripts for Phytopathology" by A. J. Riker, published in PHYTOPATHOLOGY 36: 953-977, 1946, as well as current issues of PHYTOPATHOLOGY.

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PREPARING MANUSCRIPTS FOR PHYTOPATHOLOGY

W. C. Price [1]

The Council of The American Phytopathological Society at the 46th Annual Meeting of the Society authorized the Editor-in-Chief, in consultation with the Editorial Board, to compile directions on preparation of manuscripts for PHYTOPATHOLOGY (5). These directions are not meant to replace but rather to supplement the paper by Riker (6) on the same subject. The chief objective is to bring authors up to date on present editorial policy.

This paper is intended to assist authors in preparing manuscripts, to help establish uniformity in usages and procedures, and to clarify editorial policy, procedures, and practices. Papers prepared in accord with the rules herein listed and the directives in the paper by Riker can be reviewed more quickly, will require less of the authors' time for revision, and will appear in print sooner than if the rules are disregarded.

It is not the purpose here to instruct authors in the art of writing technical articles. Authors may gain insight into this art by reading one or more of the articles cited in the bibliography attached hereto. Other pertinent publications are cited by Riker (6). Authors are urged to read Riker's paper before starting preparation of a paper intended for submission to PHYTOPATHOLOGY.

Submission of a paper for publication in PHYTOPATHOLOGY implies that it is based on original research not previously published, is not under consideration for publication elsewhere, and, if accepted, will not be published elsewhere in the same form without the consent of the editors. The editors will accept review papers provided such papers develop a new concept, hypothesis, or theory, or otherwise integrate the science of plant pathology (4). Summations of the literature are not acceptable. A paper is not acceptable unless one of the authors is a member of the American Phytopathological Society, invitational papers excepted.

There is no restriction on length of articles acceptable for PHYTOPATHOLOGY. The editors prefer that the report of a particular phase of a research problem be complete rather than broken into several short articles. This is not meant to imply that articles should be verbose; conciseness is essential. On the other hand there may be as much reason for condensing a short article as for condensing a long one.

GENERAL COMMENTS ON AIMS.—The general aims of scientific writing are communication of significant factual information and exposition of theory. The language should be as simple, clear, direct, and concise as the author can make it. Visual images and analogies should be used sparingly unless the author is certain that a direct description of a finding would be outside the experience or comprehension of the reader.

A considerable amount of basic background knowledge in the possession of the reader may be assumed by the author. Technical jargon, properly used, saves time for readers if comprehensible to them (2) and saves valuable space in a journal. However, PHYTOPATHOLOGY is read and used by many to whom English is not native and to whom looseness of language would result in confusion or misunderstanding. Consequently, ambiguity of words and grammar should be avoided. Words should be chosen with care, so that the specific word with a precise meaning, universally accepted, is used instead of a loose approximation. There is little place in scientific writing for the "common usage", *per se*. If the "common usage" meaning of a word differs from that etymologically derived, another word, more precise in meaning, should be substituted.

Although it has been emphasized that writing appropriate for PHYTOPATHOLOGY should be precise and concise, it should not be so cryptic as to be ambiguous. The author is responsible for the final interpretation of his findings. The reader should never be left in the quandary of being forced to choose between possible meanings. If a finding has more than one possible meaning, the author should say so. There is more value in stating a doubt than permitting possible misinterpretations. If, however, the substance of an article resolves into multitudinous qualifications, many "possibles", "probables", "perhaps's", or "it is likely that's", it is doubtful that the material was sufficiently "digested" for presentation or that the experimental evidence was sufficiently extensive for publication.

Speculation has provided the stimulation for much great achievement, but it is not the achievement itself; it is, rather, a tool. There is a place for imaginative speculation in phytopathology but it should not be a substitute for solid experimental work.

CHRONOLOGY OF EDITORIAL PROCEDURES IN HANDLING MANUSCRIPTS.—An article received in the editorial offices is acknowledged by postcard to the author and sent to an editor or a specialist for review. Illustrations accompanying the manuscript help the reviewer understand and judge acceptability of the article; authors should therefore supply additional copies of the illustrations for the reviewer's use. Use of unmounted copies or reproductions for review purposes lessens the danger that original copies will be damaged by sending through the mails.

The anonymity of the reviewer to the author is maintained by return of an unsigned copy of criticisms and recommendations. The manuscript is marked for press composition and returned to the author with specific recommendations for revision, if revision is needed. If costs of engraving illustrations exceed

[1] I am much indebted to Wilson S. Borland and Jane E. Borland who helped organize the paper, wrote the first draft of several sections, and reviewed the semi-final draft of the entire paper. I am also much indebted to members of the Editorial Board for many valuable suggestions, which have added materially to the clarity and usefulness of the paper.

PHYTOPATHOLOGY's allowance, the author is informed that he will be billed for the excess.

On return from the author, the manuscript is again scrutinized; if found acceptable, the date of acceptance is indicated. The manuscript is then sent to the printer to be set in type.

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Articles are published in the order in which they are accepted, except that physical make-up of the Journal may require use of a paper bearing a later date of acceptance than papers to be published in a subsequent issue.

Only when a sufficient number of galley proofs have been returned from authors are they used to make a dummy from which the printer can arrange type to fit the page dimensions of PHYTOPATHOLOGY. Consequently, authors should return galley proofs within 48 hours. Failure to do so may delay publication of the article a month or more or, even worse, delay publication of an entire issue. Present policy is not to accumulate a backlog of galley proofs but to publish articles as promptly as feasible. Implementation of this policy requires cooperation from authors in returning galley proofs.

PHYSICAL PREPARATION OF THE MANUSCRIPT.—*Format.*—Manuscripts should be typed double spaced on only one side of white bond paper, preferably 8½ x 11 in. in size. Use a heavy black ribbon so that the typing is clear, distinct, and easily read. Leave 1-in. margins to the right, left, top, and bottom. All typed material, including footnotes, literature citations, table headings, footnotes to tables, and legends for illustrations, should be doubled spaced. Double space everything. Space between lines and at the margins is needed for the editor to note corrections and instructions for the printer. Submit a top, or ribbon, copy of the manuscript and a clean carbon copy on bond paper; the author should retain a copy as a safeguard against loss. Many reviewers object to reading a carbon copy prepared from well-used carbon paper on thin transparent paper (e.g. onion skin).

General structure and arrangement.—Papers longer than a page or 2 should be divided into sections with appropriate headings; this does not apply to Phytopathological Notes. A table of headings, with the rank of each heading indicated by indentation should be included; such a table is helpful to both authors and editors in arranging the paper. The rank of each heading is indicated as follows: 1) First rank: underline the first letter with 3 rules and the other letters with 2 rules; this indicates large capitals and small capitals. 2) Second rank: underline the entire heading with a single rule to indicate italics. Capitalize only the first word and all proper names. 3) Third rank: underline with a single rule as for second rank headings and precede by a numeral and a parenthesis, as in the following paragraphs.

A good arrangement of a paper is 1) Summary, 2) Introduction, 3) Review of literature, 4) Materials and methods, 5) Results, 6) Discussion, and 7) Literature cited. Other arrangements may, however, be more suitable for particular papers.

Principal parts.—1) *Summary.*—Each main article in PHYTOPATHOLOGY should start with a Summary, which does not exceed 3 per cent of the paper. Although based on the paper, the Summary is not strictly a part of the paper but independent of it. It should convey the content of the paper, draw attention to all new information and to the main conclusions. It should be factual. It should not be a mere description of the contents of the paper. Such phrases as "a new species of Phytophthora is described" and "a description is given of a new method of assaying tobacco mosaic virus" should be avoided. The Summary may be written so that it can also be used as an abstract for publication in Biological Abstracts. To indicate the end of the Summary, draw a line below it, or type one in.

2) *Introduction.*—Begin with an introduction that is brief and to the point, one that states the problem and the reason for presenting the findings. The reader who must scrutinize 1 or more columns of type to discover the purpose of a paper may become bored and seek more stimulating articles for perusal.

3) *Review of literature.*—Not all papers require a section devoted to review of literature; authors may prefer to confine their comments about the literature to the Introduction, Discussion, or other sections. If a review of literature is required, it should follow the Introduction and precede a section on Materials and Methods. Generally, long literature reviews are to be avoided, especially if the reader can be referred to a published review of essentially the same literature. However, the relevant work of others should not be ignored.

4) *Materials and methods.*—This section should include those materials and methods generally used in the experimental work to be reported. They are described once, and once only. Special methods or materials that apply only to a particular experiment should be mentioned where the procedure of that experiment is outlined.

5) *Results.*—The main body of the paper should be divided by headings and subheadings into sections that make it easy for the reader to find details in which he is particularly interested. It should be organized according to a logical sequence, not necessarily the order in which the experiments were carried out. The heading "Results", "Experimental results", or "Experimental work" may be used or omitted; if omitted, subheadings are necessary to separate this section from Materials and Methods and Discussion.

From a minimum of words, the reader should get

a clear picture of the reasons for doing the experiment, the procedures followed, and the results obtained. If interpretation of the results is obvious, do not waste words in interpretation.

Avoid giving the results of an experiment in tables and charts and again in the text. If the results can be clearly summarized in a few words in the text, do not present them also in tabular or chart form. Charts are cheaper to reproduce than tables and usually are easier to read and understand.

Detailed presentation of data in tabular form is undesirable; the data should be reduced to means and an appropriate measure of their variability, such as standard deviation. Avoid "notebook" reporting. On the other hand, do not expect all your readers to be statisticians; present the data in a simple form that is understandable to the average reader. Each time a table or a chart is assembled, the author should ask himself a question: What is the purpose of this table, or chart? Is it to show how much work was done or is it to present the findings in a form most comprehensible to the reader? Inexperienced authors, especially, seem to feel that major conclusions should be buttressed by innumerable tables of detailed data. But a table is no better than the integrity of the worker. Summarize the data into brief, meaningful tables or charts that are easily and quickly comprehended by the reader. Do not expect or require the reader to make the analysis for you.

Avoid unnecessary columns. For example, if the total number of plants is given in one column and the percentage infected in another, there will be no need to present the number of plants infected and the number not infected in additional columns.

6) *Discussion.*—The author may wish to relate the experimental findings to major problems in the field of work. The heading Discussion is appropriate here. However, not all papers require a Discussion. Do not use the Discussion for recapitulating or summarizing the material presented under Results.

7) *The name and address of the institution* where the work was done should follow the Discussion and precede Literature Cited.

8) *Literature cited.*—When 5 or more references to literature are cited, the references should be brought together at the end of the manuscript. The form of the citation is that recommended by the A.I.B.S. Committee on Publication Problems (7). If in doubt, refer to Literature Cited in recent issues of PHYTOPATHOLOGY. Literature citations are considered in more detail in a later section of this paper.

Abbreviations.—Units of time (e.g. minutes) are not abbreviated. Units of measurement (e.g. in.) are abbreviated when such abbreviation will not lead to confusion. Abbreviations for the plural are the same as for the singular. Preferred abbreviations for PHYTOPATHOLOGY are (note presence and absence of periods):

A	for Angstrom
a.m.	for ante meridian
C	for Centrigrade
cc, or cm^3,	for cubic centimeter (a measure of volume, not a liquid measure)
cm	for centimeter
cm^2	for square centimeter
cu.	for cubic
ED_{50}	for effective dose for 50 per cent
ft.	for feet
G	for gravitational force
gal.	for gallon
g	for gram
in.	for inch
kg	for kilogram
l	for liter (may be spelled out to avoid confusion with numeral 1)
lb.	for pound
LD_{50}	for lethal dose for 50 per cent
LSD	for least significant difference
log	for logarithm
M	for molarity
m	for meter
m^2	for square meter
mm	for millimeter
mm^2	for square millimeter
mg	for milligram
ml	for milliliter
μl	for microliter
μ	for micron (10^{-3} mm)
mμ	for millimicron (10^{-6} mm)
μg	for microgram (10^{-6} g)
$\mu\mu$g	for 10^{-12} g
N	for normality
no.	for number
ppm	for parts per million (previously written p.p.m
p.m.	for post meridian
sq.	for square
rpm	for revolutions per minute
/	for by, to, or in (e.g. 2 lb./100 gal.)
*	for significant at 5 per cent level
**	for significant at 1 per cent level

Numerals.—Numerals are written out in titles, when beginning a sentence, and in direct quotations. Elsewhere arabic cardinal numbers are used.

For values less than 1, 0 should be inserted before the decimal point (e.g. 0.5, not .5). Numerical values involving many zeroes may be abbreviated thus: 2.47×10^6 instead of 2,470,000; 3.5×10^{-5} instead of 0.000035. The number of significant figures should reflect the accuracy of the results. The figures 5, 5.0, and 5.00 indicate different degrees of precision. It is a rare paper in PHYTOPATHOLOGY that requires more than 1 decimal place (e.g. 56.3) for original data.

Hyphenation.—Use hyphens only when clarification demands their use.

Footnotes.—Footnotes should be avoided whenever possible, since they are distracting and costly. References to footnotes are indicated by superior arabic numerals numbered successively throughout the paper.

Each major article, but not Phytopathological Notes, should include a footnote to the title, "Accepted for

publication", followed by a space for insertion of date of acceptance; this is referred to by placing the number 1 as a superscript at the end of the title. Additional footnotes to the title can follow as separate paragraphs without additional superscripts.

When the only footnotes in the paper are those to the title and the author's name, they may be typed immediately below the author's name. When additional footnotes occur at various places in the paper, use a single sheet of paper on which the title and author's name are followed by all the footnotes in numerical order; the Summary may follow on the same page or the next one. Use several sheets if footnotes are too voluminous to go on a single sheet. The typing should be double spaced. Do not type footnotes between ruled lines immediately below the line of type in which reference to the footnote is made. Do not type them at the bottom of the page on which the reference is made. Indicate by number on the left-hand margin of the text the point at which the footnote is to be inserted.

Binomials.—The first time the common name of a plant or a plant pathogen is mentioned, it should be accompanied by its Latin binomial or trinomial and the correct authority; e.g. *Ustilago bulla'a* Berk. var. *macrospora* (Farl.) G. W. Fisch., *Cladosporium cucumerinum* Ell. & Arth. The binomial or trinomial should be underlined for printing in italics, except in titles and in Literature Cited. Names of authors of plant parasites should be abbreviated as recommended by Stevenson (8). Whenever 2 or more authors' names are involved the final connective should be the ampersand, "&", or the Latin "et" and not the English "and"; e.g. *Helminthosporium sativum* Pam., King & Bakke; *Selenophoma everhartii* (Sacc. & Syd.) Sprague & A. G. Johnson. The generic name should be spelled out only the first time it is used unless subsequent use of an initial would lead to confusion.

Nomencla'ure for chemical compounds.—It is required that the active ingredients of fungicides or other pesticides designated by proprietary trade or code names be identified by a chemical name following accepted rules of nomenclature, as exemplified in Chemical Abstracts. The percentage active ingredient should be declared when less than 100 per cent. Authors should note that the names given by the manufacturer on labels may be incomplete or ambiguous and that the name should be checked for accuracy before submitting a manuscript. Other chemical names likewise should follow the approved usage. It is recommended that authors consult a paper by S. E. A. McCallan and Lawrence P. Miller, Chemical names for active ingredients of fungicides, to be published in a forthcoming issue of PHYTOPATHOLOGY.

TABLES.—Each table should be typed on a separate sheet, not on the same page as the text. Indicate on the text proper the approximate point at which the table is to be inserted.

Format.—Tables are set generally in 8-point type, 1 column wide (3 in.) or page width (6-3/16 in.); when preparing tables, it is well to keep in mind that 52 typewriter spaces are roughly equivalent to 3 in. of 8-point type. Very large tables must be set in 6-point type, which is difficult to read, or by the photographic process.

The general format of a table is as follows: 1) The table should have a heading, beginning with the word "Table" and the appropriate arabic numeral, followed by a period and a dash. The heading should be a brief, specific description of the contents of the table, not a conclusion drawn from the data. Only the first word and proper names are capitalized; all figures are in arabic cardinal numbers. Do not place a period after the title. The title should be underlined (one rule) to indicate to the printer that it is to be set in italics. Do not put a second rule under names of species; one rule under the entire heading is enough. 2) Immediately beneath the heading use a double line to separate the heading from the body of the table. 3) Columnar headings are in roman type unless the use of italics will clarify specific meanings. 4) Single horizontal rules may be used to indicate relationship of rows. If italics are desired in any portion of the table body, this should be indicated in pencil in the margin since printer's "rules" and italic markings are indistinguishable on the typewriter. No vertical rules are used to set off columns in the printed text, but the typist may use them to guide the printer in spacing the columns. A single line should follow the main body of the table. 5) Footnotes appear beneath the bottom rule of the table and are indicated, both in the body of the table and at the footnote itself, by superior, lower-case letters, a, b, etc.

Content and use of tables.—Tables should be comprehensible without reference to the text. They should be used to exhibit significant data when this form will make the clearest and most concise method of presenting information. They should not be mere duplications of field or laboratory notebook data. Carefully selected significant data will contribute much more to the reader's understanding of the writer's exploration of the problem and experimental results than a more detailed, complicated table.

The printing of tables entails the most tedious and costly kind of typesetting. Frequently, the same basic information can be presented more clearly and more vividly in graphic form, using graphs, bar charts, or other forms. These can be reproduced, by a photographic process, far more cheaply than can tables. Often detailed tables can actually be translated into a few sentences of text without loss of valuable information. A table should be a "shorthand" method of presenting important findings, not a way of expanding what could otherwise be expressed in a few brief sentences. It should be predigested for the reader.

In the preparation of a paper, a writer would do well to ask himself the following questions: 1) Are tables needed? 2) Will they be the most concise method of presenting data? 3) Can the information be communicated without important loss in a few sentences of text? 4) Will a graph or chart be as good or better?

Consider carefully the form of the table, with column and page dimensions in mind. Consult Riker (6) for details regarding style and form.

PREPARING ILLUSTRATIONS.—Illustrations (or "Figures") used in PHYTOPATHOLOGY are of 2 kinds: 1) photographs, reproduced by half-tone cuts, and 2) line drawings, charts, graphs, etc., reproduced by zinc cuts. A clear illustration may be the most important part of an article; a poor illustration is worse than useless—it wastes valuable space.

Points to remember.—Make illustrations the exact size you wish used or not more than 50 per cent larger; better results are obtained for reproduction when the engraver can reduce rather than be forced to enlarge a photograph or a drawing. Avoid oversize illustrations that require special packaging and cause extra work for the editorial office and for the printer.

The size of the page face in PHYTOPATHOLOGY is 6-3/16 in. by 8½ in., with a division into 2 columns 3 in. wide by 8½ in. tall. Your illustrations should be of such proportions that space is not wasted: i.e., final size not less than 3 in. wide (column width) or 6-3/16 in. (page width).

When planning a full-page figure, allowance for a legend should be made; this must be deducted from the total height of 8½ in. Allow ¼ in. for the first line of legend, which should total not more than 115 typewriter spaces, and ⅛ in. for each successive line. For example, 4 lines of legend will require ½ in. and the ratio of the figure must be 8 x 6-3/16 in.

Photographs should be printed on glossy paper, be contrasty in character—light against dark or dark against light—and as sharp as possible. The screen reproduction process tends to soften outlines and reduce contrast. An original photograph that is dull in contrast and soft in line quality reproduces as a gray blur.

When a number of illustrations are essential to an article, they should, where possible, be combined to form panels with the sections clearly lettered. This method has several advantages: it simplifies editorial make-up; it improves the appearance of the article; and it is economical both in use of space and in cost. For example, an article calls for 6 small illustrations; if these are used separately, 6 plates must be prepared, each costing the basic charge and more; if the 6 are combined in 1 plate, captioned Fig. 1-6, there is but 1 basic charge. To illustrate further, the Society allows $16 an article for illustrations; half of any excess is paid by the author. In the above noted example, a full page plate can be made within the cost allowance of the Society; if the 6 illustrations were used separately, the Society's allowance would pay the basic charge on less than 3 plates. An engraving 1 inch square or smaller costs a minimum of $6; the cost per unit decreases as the size increases.

In combining illustrations, photographs and line drawings should be handled separately, since different methods are used to reproduce them. Number all figures consecutively with arabic numbers whether they occur singly or in groups. When in groups, they should be numbered consecutively from left to right and top to bottom, irrespective of the order in which they are mentioned in the text. Use capital letters for designating component parts of a figure. An illustration with numerals or capital letters is less likely to be inverted accidentally in the editorial office or the printing plant. Printed numerals and letters should be securely pasted on the illustration, or the numerals and letters may be inked directly on the photograph or line drawing. If letters and numerals are not securely fastened to the illustration they may fall off and their absence not be noticed until the illustration appears in print.

Graphs.—Line illustrations—camera lucida drawings, charts, graphs—should be prepared in black India ink, on good paper, illustration board, or tracing cloth. All lettering should be large and clear, preferably "guided" letters such as are drawn with Wrico or other guides. Line illustrations make excellent small reproductions (3 in. width, or smaller), provided the original lettering is large and clear. For best reproduction, authors should submit original drawings rather than photostats or other prints. Consult Riker (6) for suggestions about size of lettering.

When preparing graphs for publication, one can profit by reading the excellent treatise published by The American Society of Mechanical Engineers (1). Proper planning of line drawings can result in appreciable saving of space in a journal. When several are used, combine them into a single plate, keeping in mind the column (3 in.) and page (6⅛ in.) widths. Fig. 1-3 of the paper by Halpin, Hanson, and Dickson (3) represents an example in which 3 graphs have been combined to fit across a page.

There are several additional points to remember. 1) Lines on 4 sides should enclose the graph in a rectangle; this is preferable to inking-in only the ordinate and abscissa. 2) Experimental points should be indicated by appropriate symbols, not merely by abrupt changes in slope of the line. The symbols used to portray points on different curves in the same graph should be distinctive; plain circles and filled-in circles, the latter slightly smaller than the former, are easy to draw and more easily distinguished than squares and triangles. Every symbol should be sufficiently large that it is not reduced to a black dot in the engraving. 3) Lettering should be black and of a size to be easily read when reduced. Spacing should be uniform. 4) Use a lettering pen that allows ink to flow uniformly and gives a line neither too thick nor too thin. 5) When drawing curves on a graph, use French curves as guides; curves drawn free-hand usually appear untidy. Coordinate rulings should usually be avoided; replace them with short "ticks." 6) If photographic reproductions of line drawings are to be submitted for publication, be sure that the lens of the camera is parallel to the paper and that the paper is flat.

Legends or cut lines.—A good illustration needs little verbal description. The legend, or cut line, should be a brief description of the illustration, containing

only essential information necessary to clarify the object or condition depicted.

Legends should be typed double-spaced on a sheet or sheets separate from the illustrations. This is essential since the legends are set in type in the printing plant whereas the illustrations themselves are handled by an engraver who may be in a different city. When authors attach the only copy of the legend to the illustration they have created additional and otherwise unnecessary work for the editorial office.

LITERATURE CITATIONS.—Accuracy and uniformity in literature citations are important for the convenience of the reader, especially one in another country. Complete data help him locate the source material in his local libraries more easily. Literature citations in PHYTOPATHOLOGY therefore follow the form recommended by the American Institute of Biological Sciences (7). The following are typical examples.

Ciccarone, A., and J. T. Middleton. 1951. Two species of Pythium recorded from Venezuela. U. S. Dept. Agr. Pl. Dis. Reptr. 35: 387.

FISCHER, G. W. 1951. The smut fungi. A guide to the literature, with bibliography. The Ronald Press Co. New York. 387 p.

TOMPKINS, C. M. 1939. Two mosaic diseases of annual stock. Jour. Agr. Res. [U.S.] 58: 63-77.

As in the rest of the manuscript, literature citations are typewritten and double-spaced. Some authors assume that because literature citations are correct it is not necessary to double-space them. This is erroneous. Frequently the citations are incorrect in arrangement, as to page numbers, or in other detail. Even if correct, they still must be marked with instructions to the printer, and space between the lines is needed for this purpose.

The heading "LITERATURE CITED" is captialized and centered on the page. References are listed numerically, with arabic numerals. They are arranged in groups 1) alphabetically according to the surname of the first or sole author, 2) alphabetically according to the surname of the junior authors; each group is arranged chronologically according to year of publication. In each citation, the proper name of the senior author is given first, followed by his initials and by junior authors' names in natural order. Only the initials of male authors' given names appear, but women's given names are spelled out. All authors' names are in large and small capitals and written out for each citation; in the examples, this is indicated by triple and double underlines and it therefore does not matter whether the names are typed in capitals or lower case. The year of publication and title follow the authorship in order. Lower case type is used in the title except for proper nouns, and no scientific names are italicized. Names of journals cited are abbreviated except when they consist of a single word, e.g. Phytopathology. When an abstract is cited the abbreviation (Abs.), in parentheses, follows the title. Volume and page numbers of citations in periodicals are written as arabic numerals and appear in the following order: volume number (underlined with a wavy line to indicate that it should be set in bold face type), colon, limiting pages separated by a dash. When each issue of a journal is paged separately, the number of the issue in parentheses should follow the volume number. Book citations differ at this point in that they include, following the title, the edition other than the first, the publisher's surname, the place of publication, and the number of pages and volumes if more than one. The citations are numbered consecutively and referred to in the text by the corresponding number in parentheses. The author can refer to a specific chapter, table, or page in the citation by mentioning the chapter, table, or page in the text of his article.

PHYTOPATHOLOGICAL NOTES.—*Contents.*—Phytopathological notes consist of short articles in which 1 or 2 points not requiring detailed documentation are stated briefly. A paper is designated as a Note partly on the basis of length, 1-4 pages of manuscript. Review and publication of Notes can, at times, be handled somewhat more quickly than for main articles. Inclusion of illustrations (if not too extensive) and of concise tables is permissible.

Format.—The format of a Note differs somewhat from that of longer articles, but the same rules otherwise apply. The title is printed in italics, with important words and proper nouns capitalized. The author's name, in natural order, follows the title and is printed in large and small capitals. Periods, but no dashes, are placed after the title and author's name. The date of acceptance of the Note is not printed. All references to literature are set as footnotes, designated by superscripts, and these together with other footnotes are listed numerically in the order in which they are mentioned in the text. They should therefore be typed together on a separate sheet. The place where the work was done follows at the end of the article and is set off by a dash. Phytopathological Notes are short enough not to require subheadings.

ABSTRACTS.—*General.*—Abstracts of papers presented at Annual Meetings of the Society and at various Divisional Meetings will be published in PHYTOPATHOLOGY, provided they meet requirements specified by the Council of the Society. Rules applicable to abstracts presented at an Annual Meeting are applied also to those presented at Divisional Meetings.

Requirements.—No more than 2 abstracts presented by an author at the annual meeting of the Society and at the annual meeting of a Division will be published at the Society's expense. In effect, this means that the Division will be charged for the excess if the total number of abstracts published by a single author exceeds 2; an author cannot appear on more than 2 papers at the Annual Meeting, invitational papers excepted. Abstracts of invitational papers are not counted in the number to which an author is limited.

Contents.—Abstracts that exceed 1300 typewriter spaces will be rejected; this is roughly equivalent to 200 words. Typewriters vary in number of characters or spaces to an inch. The most commonly used,

elite type, has 12 characters to each inch. For this special case, abstracts will be limited to 108 linear inches of type.

The abstracts must be in clear, concise English and contain no tabular data, either in the form of tables or running text. Only unpublished information may be included. Papers that report progress, disease occurrence, routine tests, or ordinary control measures will not be accepted unless new and significant developments are clearly indicated. Literature citations should not be included in the abstract.

Invitational papers.—Abstracts of invitational papers, or those submitted by other Societies for inclusion in joint programs, are subject to the same rules as other abstracts except that the rules regarding unpublished information, membership in the Society, and limitation on number of abstracts will be waived.

Format.—The entire abstract should consist of a single paragraph. The title is run as a sidehead in italics; only the first word and proper nouns are capitalized. The title is followed by the first author's name, in reverse order, and by junior authors' names in natural order. Authors' names are in large and small capitals; periods, not dashes, follow the title and authors' names.

OBITUARIES.—*Length.*—Generally, an obituary for publication in PHYTOPATHOLOGY should be limited to 1 printed page. If, however, more than a page is required an effort should be made to fill an exact number of pages. For example, an obituary that is long enough to fill 1½ pages will be cut to fit on a single page.

In estimating length, allow for 6000 characters, including spaces, per page. Space for a title will use 150 characters and that for an illustration will use 1580 characters. Therefore, allow for 5850 characters for page 1 if an illustration is not available, and for 4270 characters if a 3 in. cut is used.

Contents.—Since PHYTOPATHOLOGY is a technical journal, the obituary should mainly concern itself with a description of the individual's career in plant pathology and with the impact of that career on the development of the science of plant pathology. Personal data to be included are: time and place of birth and death, education, degrees received, positions held, membership and offices in scientific societies, and special appointments. Not to be included are personal experiences that have no relationship to plant pathology or allied sciences. Exceptions are personal experiences that influenced a major decision with respect to the individual's career in science, or extra-mural activities (civic, community, or volunteer services) related to the individual's scientific interests.

Style.—The style should be the author's own. The editors, however, reserve the right to edit for grammatical construction, brevity, and clarity and to delete passages otherwise acceptable if such deletion is required to attain a proper length.

Illustrations.—If possible, provide a sharp glossy print, which can be reduced to fit a single column (3 in. wide). A portrait by a professional photographer will ordinarily reproduce best, but "snapshots" can be used if a portrait is not available.

Bibliography.—Space in PHYTOPATHOLOGY is not available for extensive bibliographies. A few of the most important papers may be cited and the citations will be printed when space is available for them.

GALLEY PROOFS.—The first printed form of a paper's text is a galley proof. This is read in the editorial office for obvious errors and misprints, and corrections are made in red pencil. One corrected copy is sent to the author, with the original manuscript. The author should read the galley proof at least twice. It is also recommended that the author ask someone unfamiliar with the manuscript to read the galley proof. Make changes only when absolutely essential, when they are required to correct errors of fact or of typography. Changes that require additions or deletions of words should be avoided; they may require the resetting of entire paragraphs in type. Engraver's proofs of illustrations should be examined for defects and initialled by the author if found satisfactory. When galley proof and manuscript are returned to the editorial office, author's corrections are transferred to the editor's copy of the galley proof for use in the final make-up of the journal.

Suggestions to authors.—Author's corrections should be made in lead pencil; this simplifies transfer to the editorial copy. Authors should also initial each sheet of galley proof as it is read; this identifies each sheet and eliminates any doubt in the editorial office that the author has read the printed text. During the author's reading, any textual corrections, additions, or deletions must be made. Remember that extensive changes necessitate resetting of type; minor changes are not charged, but if a number of lines must be reset or added, a charge is made to the author for each line that is reset.

Tables should be checked closely for both form and content. The typesetting involved in tables is tedious and difficult; tables are more susceptible to error than straight text.

No article will be used until the author's copy of the galley proof has been returned. It is not necessary for the reprint order to accompany the galley proof. The Editor-in-Chief does not send reprint orders to the printer until page proof is available. Consequently, delay of 2–3 weeks in returning the reprint order is permissible.

REJECTION, REVISION, REWRITING.—*Most frequent reasons for rejection.*—The subject matter of PHYTOPATHOLOGY is plant pathology. Papers that do not deal with plant pathology will be promptly rejected. For example, a method of measuring internal temperature of a plant might be useful to plant pathologists, but a description of the method or its development would not make a suitable paper for publication in PHYTOPATHOLOGY; a description of the method and its application in studying a disease process would be suitable.

Papers that lack adequate experimental evidence to support the author's conclusions will likewise be rejected. Sometimes the author has sufficient evidence

but fails to present it in a convincing fashion. This does not mean that his conclusions should be buttressed by innumerable detailed tables. The author should contrive to summarize his data in a brief, readily comprehensible form or otherwise indicate to the reader that there is substance as well as theory in his report.

Papers about trivial matters, such as new strains of pathogens, new insect vectors, or new hosts of pathogens will be rejected unless they are brief and to the point. They should point out the significance of occurrence of the new and the difference between the new and the old. They should not include detailed descriptions of methods and procedures nor of symptomatology in various plants.

Papers that report routine tests or those that constitute mere reports of progress will be rejected.

Papers that are poorly written, incoherent, or much in need of condensing will be rejected. Many poorly written papers are comprehensible to one who will take the time to study them but a good paper should be comprehensible on first reading.

Common reasons for recommending revision.—1) *Incoherence.*—Authors may be asked to revise a paper because the purpose of the paper and the reasons for doing the work are not made clear. If the reader knows the author's purpose, he is in much better position to follow the author's logic on first reading. They may also be asked to revise a paper because the methods and experimental procedures are not presented coherently. The reader should not be forced to extract this information from a mass of verbiage; he should get it on first reading. Papers lack coherence also when illogical or when the logic is difficult to follow. An illogical presentation results when the author assumes, albeit unconsciously, that the reader knows what is in the author's mind and fails to put complete thoughts down on paper.

2) *Prolixity.*—Extensive and detailed reviews of literature, experimental procedures and results given in entirely too much detail, duplication of material in tables, charts, and text, and just plain wordiness are common examples of prolixity to be avoided. To overcome these faults the editors can do little except recommend revision to attain conciseness, or function as rewrite men.

When a manuscript is submitted to a reviewer, a request for an answer to the following questions accompanies the manuscript: 1) Does the paper constitute a contribution to phytopathological literature? Has essentially the same material been published elsewhere? 2) Are the data and conclusions presented clearly and concisely? Do the data support the conclusions drawn? 3) Are there errors of fact, interpretation, or calculation? 4) Should the paper as a whole, or any parts of it, be condensed or expanded? If so, which parts and to what extent? 5) Do the illustrations contribute to an understanding of the paper? Are any of them unnecessary? 6) Is the tabular material complete and in good form? 7) Is material in tables duplicated in illustrations or text? Should any tables be omitted? 8) Have all references been cited in the text? Should additional references be cited?

The author should profit by asking these questions about his own manuscript before he submits it for publication.

Rewriting.—Editors are not infallible but they make an honest and serious attempt to advise authors on ways to improve papers. The author should not ignore their recommendations. Even when the editor's recommendation cannot be accepted, the recommendation should suggest to the author a need for improvement. The Editor-in-Chief may be forced to reject a paper if the author does not correct his paper accordingly or justify the original construction. Final responsibility for an article is the author's. The editors' responsibility is to the Society; their duty is to maintain high standards for PHYTOPATHOLOGY, high standards in English composition as well as in scientific methods.

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EFFECTIVENESS OF SEED TREATMENT FOR CONTROLLING ANTHRACNOSE AND GUMMY-STEM BLIGHT OF WATERMELON[1]

Harvey W. Rankin[2]

SUMMARY

Watermelon seed treatments in general use did not completely eradicate *Colletotrichum lagenarium* (Pass.) Ell. and Halst., or *Mycosphaerella melonis* (Pass.) Chiu and J. C. Walker. Mercuric chloride gave the best control of the 7 materials used. Seed disinfectants were more effective as solutions or suspensions than when used as dry dust treatments. Seedling infection by both *C. lagenarium* and *M. melonis* occurred at, or a little below, the ground level. Attempts to isolate *C. lagenarium* from the interior of seeds harvested from melons with heavy surface infections failed. The lesions did not extend through the rind. Melons which have split in the field may produce infected seed. The seed-saving process offers the most likely place and time for seed infection by both *C. lagenarium* and *M. melonis*. Attempts to isolate *C. lagenarium* and *M. melonis* from seed lots which had produced high percentages of diseased seedlings were unsuccessful with *C. lagenarium*. *M. melonis* was isolated from only 1 seed. Many other species of fungi and bacteria were isolated in these attempts. When uncontaminated seed was removed directly from melons, inoculated with pure cultures of either of the fungi and incubated for several days, both *C. lagenarium* and *M. melonis* were recovered without difficulty. The critical invasion time for seed inoculated with *C. lagenarium* and kept under favorable conditions is 24 hours. For seed inoculated with *M. melonis*, it is 12 hours. Stained sections showed *C. lagenarium* invading the epidermal layer of palisade cells and the sclerenchyma layers of the seed coat. Occasional strands of mycelium were observed extending into the parenchyma and inner epidermis. *M. melonis* invaded the epidermis and sclerenchyma layers of cells and often extended into the parenchyma and inner epidermal layers. Pycnidia were found forming in the outer epidermis and pycnidia or sclerotial-like bodies were observed in the sclerenchyma layers. Attempts to isolate *C. lagenarium* from the seed cotyledons and embryo gave negative results. *M. melonis* was isolated from cotyledons and embryo in a high percentage of cases.

Seed treatment has not given watermelon growers satisfactory control of anthracnose, caused by *Colletotrichum lagenarium* (Pass.) Ell. and Halst., or gummy-stem blight, caused by *Mycosphaerella melonis* (Pass.) Chiu and J. C. Walker. The work reported here was begun in 1950 to study the relative effectiveness of various seed treatments and to determine why the recommended materials in use are unsatisfactory.

Gardner (6), Chiu and Walker (3, 4), and others have done extensive work on *C. lagenarium* and *M. melonis*, but have never definitely established that either of the pathogens is seed-borne. Parris (7) and Ellis (5) stated that both *C. lagenarium* and *M. melonis* are carried by seed, and Wiant (10) said that *M. melonis* is seed-borne. No experimental evidence was offered for these statements. Walker (9) reported that *C. lagenarium* has not been isolated from cucurbit seed though there is much circumstantial evidence that it may be seed-borne; and *M. melonis* is said to be seed-borne but no experimental proof has been offered. Butler and Jones (2) stated that there is no substantial evidence that *C. lagenarium* is carried by watermelon seed.

MATERIALS AND METHODS. — All seed treating materials were secured from commercial sources and assumed to be in good condition. Part of the seed, 4 lb., was bought from a seedsman; its source is unknown. Seventy-five lb. of seed, Blacklee variety, was harvested from watermelons grown at the Coastal Plain Experiment Station. These seed were separated by hand from healthy melons, washed in running water, and dried within a few hours. No fermentation of the seeds and pulp was included in the process.

Check plantings of each seed lot were made in soil in a greenhouse. In no case did any of the resulting seedlings develop lesions caused by either *C. lagenarium* or *M. melonis*. The average seedling emergence of these plantings was 86 per cent.

All soil used for seed plantings in greenhouse benches was steam sterilized.

The culture medium was made by putting the contents of a No. 2 can of green beans through a blendor, making it up to 2 liters with water, and adding sufficient agar to give a 2 per cent solution. This medium gave much better spore production than did potato dextrose agar.

Seeds to be treated were kept in spore suspensions of either *C. lagenarium* or *M. melonis* at room temperature (80–90°F.) for 48 hours. This simulated part of the process commonly used by commercial seed producers. The seeds were dried, divided into equal lots by weight and exposed to one or another of the following treatments: 1) bichloride of mercury ($HgCl_2$), 1–1000 for 5 min., washed 10 min. and dried; 2) Semesan (hydroxymercurichlorophenol), 1 part to 300 parts seed, by weight; 3) 2 per cent Ceresan (ethyl mercury chloride), 1 part to 250 parts seed, by weight; 4) Arasan (tetramethylthiuramdisulfide), 1 part to 250 parts seed by weight; 5) Phygon (2,3-dichloro-1, 4-napthoquinone), 1 part to 200 parts seed,

[1] Accepted for publication July 9, 1954.
Contribution of the Department of Plant Pathology, Georgia Coastal Plain Experiment Station. Published with the approval of the Resident Director as Journal Series Paper Number 29.

[2] Plant Pathologist, Georgia Coastal Plain Experiment Station, Tifton, Georgia.

TABLE 1.—*Infection of watermelon seedlings grown from seed inoculated with C. lagenarium or M. melonis at Tifton, Georgia*

Treatment	*C. lagenarium* Plants emerged	*C. lagenarium* Plants diseased	*M. melonis* Plants emerged	*M. melonis* Plants diseased
	No.	per cent	No.	per cent
1. $HgCl_2$	132	1.5	97	6.2
2. Semesan[a]	157	23.6	90	43.3
3. 2% Ceresan[a]	167	16.8	95	21.1
4. Arasan[a]	164	18.9	88	26.1
5. Phygon[a]	159	16.4	107	19.6
6. Spergon[a]	159	28.3	98	26.5
7. Checks	153	43.1	94	42.6

[a] Dust treatments.

by weight; 6) Spergon (tetrachloro-para-benzoquinone), 1 part to 200 parts seed, by weight; 7) checks, seeds untreated.

The inoculated, treated seeds were planted in soil in a greenhouse bench. Three plantings of 250 seed per treatment were made of seed inoculated with *C. lagenarium* and 2 plantings of 150 seed per treatment were made of seed inoculated with *M. melonis*. Untreated, but inoculated, seed were planted as a check. Data were taken within the first 4 days after seedling infection appeared, to prevent secondary infections from being included in the records.

EXPERIMENTAL RESULTS. — Seeds inoculated with either *C. lagenarium* or *M. melonis* and planted in soil in a greenhouse, produced infected seedlings. Both pathogens produced similar symptoms in these seedlings. Lesions occurred on the young plant stems at the ground level, often girdling them and causing the death of the plants. Signs of the pathogens were commonly found in the lesions where ascervulae of *C. lagenarium* or pycnidia of *M. melonis* were easily observed with a hand lens. Seventy-five diseased seedlings were taken to the laboratory and the pathogens were reisolated without difficulty.

Other lots of inoculated seed were sent to the Watermelon Investigation Experiment Station at Leesburg, Florida; the Clemson College Truck Experiment Station at Charleston, South Carolina; and the Georgia Experiment Station at Experiment, Georgia.[3] Results obtained at these stations varied only slightly from those in Table 1.

Study of Table 1 shows that the relative effectiveness of the different seed treating materials was about the same for both pathogens. Bichloride of mercury was the most effective disinfectant used, and it was the only material used as a wet or dip treatment. This suggested that the other materials might be more effective if used in the form of solutions or suspensions. To test this, 3 representative materials from the first tests, plus Dithane D-14 (disodium ethylene bisdithiocarbamate) were tried. Seeds inoculated with *C. lagenarium* were treated for 5 minutes and planted without rinsing; 400 seeds from each treatment were planted.

[3] The author wishes to thank G. K. Parris, W. M. Epps, and B. B. Higgins for help and interest in this work.

TABLE 2.—*Infection of watermelon seedlings resulting from inoculated seed when each treatment was a 5-minute dip*

Treatment	Plants emerged	Plants diseased
	No.	per cent
1. $HgCl_2$, 1–1000[a]	234	2.1
2. Arasan, 1–125[a]	282	4.6
3. Dithane D-14, 1–100[a]	309	7.1
4. Spergon, 1–100[a]	280	35.7
5. Check, water soak	240	54.2

[a] Gm./cc., for dry materials; cc./cc., for liquids.

Tables 1 and 2 indicate that dip seed treatments are more effective than dust treatments. A comparison of untreated checks in Tables 1 and 2 shows that the lot of seed used for data of Table 2 contained a higher percentage of infected seed than that used for data of Table 1.

The percentage emergence of seedlings shown in Tables 1 and 2 is much lower than that of noninoculated seed lots. For noninoculated seed, emergence was 86 per cent, while the seedling emergence of checks in Tables 1 and 2 was 60–61 per cent, indicating the probability of pre-emergence damping off or seed rot in the latter.

Fruit invasion by C. lagenarium and M. melonis.— Seed treatment is usually based on the theory that surface-borne organisms are eradicated by the action of disinfestants. When a number of disinfestants are used and fail to eradicate the pathogen completely, then infection rather than infestation of the seed probably is involved.

A general opinion expressed by investigators is that *C. lagenarium* is a watermelon-rotting pathogen (7) and that a great part of the losses sustained by shippers is due to this fungus. Thus, it is sometimes assumed that the fungus can penetrate a melon and accomplish seed infection. Observations during the past 3 years do not support this assumption. Ripe melons which had lain in the field for 6 weeks after the normal harvesting period and which had become extensively encrusted with lesions due to *C. lagenarium* showed no sign of internal rotting. The lesions never exceeded a depth much over ¼ in., and the area beneath the surface was of a pithy appearance with a tendency to draw away from the remaining healthy rind. Some of these melons were opened in the laboratory and their seeds picked out with sterile forceps and plated on bean agar. Neither *C. lagenarium* nor any other fungus was recovered from such seeds.

Mycosphaerella melonis may cause fruit rot, but it is generally a foliage and vine disease. Wiant (10) reported that he had never found it causing rot in shipments of domestic melons, though he did observe it rotting melons in 1 shipment from Cuba.

Rotting of melons was found associated with *C. lagenarium* lesions in many cases, but invariably other fungi, accompanied by bacteria, were also found there.

TABLE 3.—*Infection of watermelon seedlings resulting from fermenting seed in C. lagenarium spore suspensions for 48 hours at 80–90°F.*

Source of inoculum	Seed lot	Percentage of seedlings infected
Leesburg, Florida[a]	1	54
Leesburg, Florida[a]	2	28
Leesburg, Florida[a]	3	43
Local	4	7
Local	5	5

[a] Isolates made from material received from G. K. Parris, Leesburg, Florida.

TABLE 4.—*Infection of watermelon seedlings resulting from fermenting seed in M. melonis spore suspensions for 48 hours at 80–90°F.*

Source of inoculum[a]	Seed lot	Percentage of seedlings infected
Leesburg, Florida	6	42
Auburn, Alabama	7	4
Ithaca, New York	8	0

[a] Isolates from material received from G. K. Parris, Leesburg, Florida; Urban Diener, Auburn, Alabama; C. W. Boothroyd, Ithaca, New York, respectively.

Fusarium spp. were the most common; *Sclerotium rolfsii* and *S. bataticola* were often found on the underside of the melons. It was not determined whether or not the bacteria generally found associated with the lesions could cause tissue breakdown alone. Attempts to isolate *C. lagenarium* from the deep lesions never succeeded.

Split melons are sometimes found in seed fields and are often harvested for seed along with sound fruit. Spattering rain or insects might introduce spores of either *C. lagenarium* or *M. melonis* into such melons where they would find congenial conditions for growth. A few growers have followed the practice of stabbing the melons of hard rind varieties with pitchforks 2 or 3 days before harvesting. This starts rind breakdown and makes seed extraction easier. This deliberate wounding also introduces many organisms, including *C. lagenarium* and *M. melonis*, into the seed-bearing parts of the melons.

Small holes were punched through the rind of sound melons and spores of *C. lagenarium* introduced through the punctures. After 6 days, the melons were opened and the fungus was found to be growing extensively and sporulating abundantly. Fifty seeds saved from these inoculated melons produced 11 per cent infected seedlings when planted in soil in a greenhouse.

The seed saving processes seem to offer the most probable place and time for seed inoculation and infection. Gardner (6) observed in 1918 that the process of watermelon seed extraction offers ample opportunity for wholesale surface contamination of the seed. In the modern process, the melons are torn to pieces by machines. Pieces of infected vines and melons commonly go through the machines and, when this happens, the whole mass of seeds and pulp may be drenched by a spore suspension. The resulting seed mass with 20–30 per cent pulp is bagged or placed in vats for 24 hours or longer where fermentation is allowed to take place. This gives time for distintegration of pulp and makes for easier separation of pulp and seed in the washing process. During the seed harvesting process, plentiful inoculum of *C. lagenarium* and *M. melonis* is often distributed through this favorable growth medium at suitable temperatures for fungus infection of the seed. Unfavorable pH, reduction of oxygen supply, or excessive competition from other organisms growing in the fermenting mass, may at times inhibit growth of pathogens. Chiu and Walker (4) showed that *M. melonis* grew over a wide pH range. Gardner (6) demonstrated that spore germination of *C. lagenarium* was retarded by reduced oxygen. Sheldon (8) proved that *C. lagenarium* grew well with reduced oxygen supply but sporulation did not take place. No information is available on the growth of these 2 fungi when in competition with other organisms.

Seed fermentation experiments.—Eight lots of seed, ranging from 2 to 8 lb. each, were used in this work. Plantings from all lots before inoculation gave only negative results for *C. lagenarium* and *M. melonis*. Five lots were fermented with spore suspensions of *C. lagenarium* and 3 lots with spore suspensions of *M. melonis*. All lots were kept in the spore suspensions for 48 hours at summer room temperature (80–90°F.). Plantings after inoculation gave the results shown in Tables 3 and 4.

The data in Tables 3 and 4 show that isolates of *C. lagenarium* and *M. melonis* varied greatly in virulence. Virulence varied with the location from which the isolate material came, age of the culture, and the host from which the isolate was obtained. It was noted that in several cases cultures of *C. lagenarium*, which were highly virulent when first isolated, lost much of their virulence after several transfers.

Attempts to reisolate pathogens from fermented seed.—Many attempts were made to reisolate *C. lagenarium* or *M. melonis* from seeds fermented in spore suspensions of these fungi, samples of which had produced high percentages of diseased seedlings. All attempts to reisolate *C. lagenarium* failed. *M. melonis* was recovered from 1 seed. Other organisms isolated in profusion from these seeds included *Fusarium* spp., *Helminthosporium* spp., *Sclerotium rolfsii*, *S. bataticola*, *Rhizopus* spp., *Aspergillus* spp., *Penicillium* spp., unidentified non-sporulating fungi, and bacteria. These results suggested that the *Colletotrichum* and *Mycosphaerella* might be outgrown and suppressed by more vigorous competitors in or on the seed. It seemed desirable to develop a technique which would eliminate the contaminants and allow the pathogens to grow freely if they had accomplished seed invasion.

Use of pure culture method in determining seed invasion by C. lagenarium and M. melonis.—Healthy melons were opened carefully to avoid external contamination. The seeds were picked out with sterile

TABLE 5.—*Infection of watermelon seeds inoculated with C. lagenarium or M. melonis and disinfected with sodium hypochlorite at 6-hour intervals*

Hours of incubation	*C. lagenarium*		*M. melonis*	
	No. of seeds tested	No. of seeds infected	No. of seeds tested	No. of seeds infected
6	11	0	9	0
12	17	0	9	4
18	17	0	9	7
24	17	4	9	5
30	17	8	9	7
36	17	9	9	9
42	17	11	9	8
48	17	15	9	9

forceps and placed in sterile culture dishes. The seeds were inoculated by mopping them with a swab which had been dipped in a heavy spore suspension of the pathogen. The inoculated seeds were incubated at summer room temperature. Every 6 hours the dishes were opened and a number of seeds removed. These were quickly dipped in 95 per cent alcohol and then placed in a 5.25 per cent sodium hypochlorite solution (Clorox) for 4 minutes. They were then plated on bean agar without washing and incubated for 7 days.

The data in Table 5 show that both *C. lagenarium* and *M. melonis* invaded the watermelon seeds and that the invasion was progressively greater with time. As invasion progressed, there came a time for each pathogen when the disinfectant used did not penetrate the seed deeply enough to inactivate it. This may be called the "critical invasion time". The critical invasion time for seed inoculated with *C. lagenarium* and kept under favorable conditions was 24 hours; for seed inoculated with *M. melonis*, it was 12 hours. The seeds showing infection produced typical colonies and sporulated profusely. No difficulty was experienced in reisolating the pathogen from any infected seed.

Extent of seed invasion by pathogenic fungi.—A short description of watermelon seed anatomy is given here for a better understanding of seed invasion. For more detail refer to Barber (1) and Fig. 1. The embryo and cotyledons are enclosed in a seed coat which is made up of several layers of different types of cells. The outer epidermis is composed of a layer of palisade cells and is 35 μ or less in thickness. Inside this lie several layers of sclerenchyma cells with thick, sinuous cell walls which are deeply pitted. Beneath these are layers of parenchyma, and finally the inner epidermis consisting of thin-walled cells.

The extent of seed invasion was determined by sectioning and staining, and by culturing parts of the seeds. Seeds that had been fermented for 48 hours in a spore suspension were hand sectioned, stained with cotton blue, and mounted in lactophenol. Mycelium was observed in the outer epidermis and layers of sclerenchyma, but it could not be determined whether or not this mycelium was that of a pathogen or of a saprophyte. The pure culture method was then used, and the seeds were incubated for 96 hours. This lengthening of the incubation period from 48 to 96 hours may seem unwarranted. It was not done to prove seed invasion but to obtain better material for study. However, some commercial melon seeds are actually subjected to 96 hours processing.

Seed coat invasion of watermelon seed inoculated with C. lagenarium and incubated for 96 hours.—The epidermis was heavily invaded by mycelium. In the sclerenchyma layers the mycelium was plentiful but not so abundant as in the epidermis. The mycelium was intracellular and of smaller diameter than that in the epidermis. It was of reduced diameter where it passed through the cell walls, thickening, as appresoria, against the cell walls where penetration was effected. Occasional strands of mycelium were observed penetrating the inner sclerenchyma layer and protruding into the parenchyma and inner epidermal layers.

Seed coat invasion of watermelon seed inoculated with M. melonis and incubated for 96 hours.—The epidermis was heavily invaded by mycelium. Occasional pycnidia were observed forming in the base of this layer against the outside of the sclerenchyma layers. The sclerenchyma layers were invaded by intracellular mycelium. Occasional sclerotial-like bodies or pycnidia were observed forming within the cells of these layers. Frequent strands of mycelium extended from the inner sclerenchyma into the parenchyma and inner-epidermal layers.

Cotyledon invasion by watermelon pathogens.—Hand-sectioning and staining of cotyledons was found to be unsatisfactory, so cultural methods were used to determine whether or not invasion occurred. Fifty seeds inoculated with *C. lagenarium* and 24 seeds inoculated with *M. melonis* were incubated 96 hours under pure culture conditions. In all cases the seed coat was removed and the undamaged cotyledons and embryos were surface sterilized for 2 minutes in 1 per cent sodium hypochlorite (1 part Clorox to 4 parts water). They were then plated on bean agar and incubated for 7 days. *C. lagenarium* failed to invade a single cotyledon, whereas *M. melonis* invaded 16 cotyledons.

DISCUSSION.—Seed treatments used in this investigation included materials generally recommended for watermelon seed in the Southeastern United States. Whereas some treatments were much better than others, none completely eradicated *C. lagenarium* or *M. melonis*. The fact that disinfecting materials gave better results when used as solutions or suspensions may be due to the sealing of the seed by the dried muscilagenous cuticle. This would give the invading pathogens a certain amount of protection. When wetted, the cuticle swells and becomes soft and probably more permeable, allowing greater penetration of the chemicals. Considering the work of Gardner (6) on dissemination of *C. lagenarium* spores and the spread of anthracnose from a few centers of infection in a field, it seems that complete exclusion or eradication of this fungus from watermelon seed would be

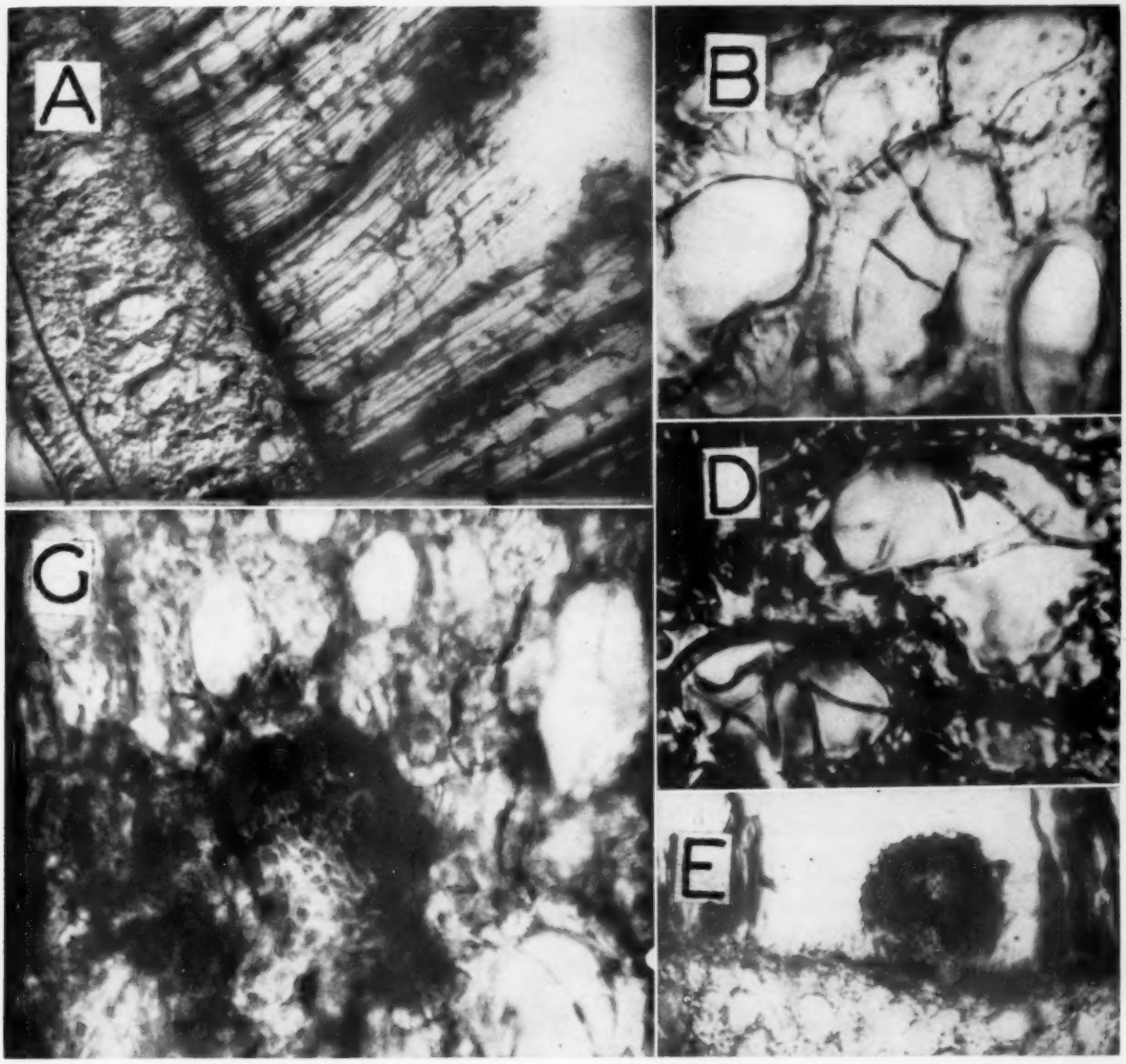

Fig. 1. Photomicrographs of cross sections of watermelon seed coat showing invasion by (A, B) *C. lagenarium* and (C–E) *M. melonis.* A) *C. lagenarium* mycelium throughout the epidermal and sclerenchyma layers; B) high power magnification of mycelium in sclerenchyma layer cells; C) *M. melonis* forming sclerotial like body in sclerenchyma cells; D) mycelium in sclerenchyma cells; E) pycnidium on sclerenchyma layer and at the base of the epidermal layer.

necessary to obtain control of the disease either through seed treatment or improved seed-saving procedure. The work reported here does not preclude the possibility of eradicating pathogens from watermelon seed by seed treatment. However, the fact that *C. lagenarium* may invade the watermelon seed coat to its innermost parts and that *M. melonis* may invade the seed coat and cotyledons and produce reproduction bodies within the seed coat, puts the problem of seed treatment in a new light. Proof has not been obtained that *C. lagenarium* never invades the cotyledons. Since mycelium is sometimes found in the parenchyma cells close to the cotyledons, it is possible that they may be invaded occasionally by *C. lagenarium.*

The procedures duplicated in this work are not necessarily typical of those practiced by all commercial seed producers, but the conditions created in the present experiments are often encountered in commercial seed processing. It is not claimed that seed producing 30–50 per cent diseased seedlings is often, or ever, produced by seed growers, though it may be. This report shows only that seed invasion may take place under existing practices and that seed infection is a source of new centers of disease in watermelon fields. A recognition of these facts and a correction of faulty practices should improve the watermelon seed situation. It should be possible to devise a

mechanical separator which would divide the seed from the pulp immediately after harvest.

Department of Plant Pathology
Georgia Coastal Plain Experiment Station
Tifton, Georgia

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CHEMOTHERAPEUTIC ACTIVITY OF UNSUBSTITUTED HETEROCYCLIC COMPOUNDS[1]

David Davis[2], Chien-Pen Lo[3], and A. E. Dimond

SUMMARY

Unsubstituted heterocycles were tested as chemotherapeutants for control of Fusarium wilt of tomato. Neither 5-membered heterocycles nor 5-membered heterocycles condensed with 1 benzene nucleus were chmotherapeutic. However, 5-membered rings condensed with 2 benezene rings significantly reduced disease. Oxygen, sulfur, and carbon were interchangeable in the 5-position as indicated by the chemotherapeutic activity of dibenzofuran, dibenzothiophene, and fluorene. The nitrogen analogue, carbazole, was not chemotherapeutic but could be reactivated with an alkyl substituent on the nitrogen. Opening the carbon to carbon bond between the 2 benezene nuclei generally resulted in a loss of activity.

Six-membered rings whether or not condensed with benzene were chemotherapeutically inactive.

The chemotherapeutic activity of benzothiazole and substituted benzothiazole compounds (4) suggest that other heterocyclic compounds might be useful in control of Fusarium wilt of tomato. So that chemical structure could be related more easily to biological activity, the investigation was confined to compounds with restricted differences. Accordingly, only unsubstituted heterocycles were examined for chemotherapeutic activity. This limited the differences among compounds to a) type of heteroatom, i.e., oxygen, sulfur, and nitrogen, b) number of heteroatoms per molecule, c) 5 vs. 6-membered rings and d) condensation with benzene rings.

Methods and materials.—Chemotherapeutic activity was measured on the Fusarium wilt disease of tomato. Bonny Best tomato plants (*Lycopersicon esculentum* Mill.) were grown in sand culture supplemented with 50 ml. of inorganic nutrient applied 3 times per week. At approximately the 6-leaf stage, 10 applications of 50 ml. of an aqueous solution of chemotherapeutant was applied over a 2-week period to the sand culture. Chemicals were applied at concentrations non-phytotoxic to tomato plants. The non-phytotoxic dose was determined by placing tomato seedlings, in the 2-leaf stage, in amber vials containing approximately 10 ml. of the chemical. Previous experience indicated that concentrations higher than ¼ the maximum dose non-phytotoxic to tomato seedlings were generally injurious to the tomato plants in the final Fusarium assay. Accordingly, most of the tomato plants in the final assay were treated with one ¼ the maximum dose non-phytotoxic to the tomato seedlings. At the conclusion of the treatment period (2 weeks) the plants were uprooted and the roots dipped in a bud cell suspension of *Fusarium oxysporum* f. *lycopersici*. Inoculum was prepared by growing the parasite on an aerated casamino acid nutrient medium. Approximately 2 weeks after inoculation, disease indices were determined. A detailed description of the chemotherapy assay and the method of disease analysis has been described by Dimond et al (5). In vitro fungitoxicity was determined by measuring growth of *F. oxysporum* f. *lycopersici* on Czapek's nutrient broth supplemented with microelements and autoclaved for 15 minutes at 15 lb. pressure. The culture was incubated 8 days at room temperature. The mycelium was then washed with distilled water and acetone (to remove the water

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The research reported here was done in cooperation with the Crop Protection Institute.
[2] Plant Pathologist, Crop Protection Institute.
[3] Research Chemist, Rohm and Haas Company.

TABLE 1.—*Chemotherapeutic activity of 5-membered unsubstituted heterocycles in the Fusarium wilt disease assay*

Compound	Concentration x10^{-3} M	Chemotherapeutic Activity Vascular Index
Thiophene	6.0	0.64
Pyrrole	0.09	0.69
Thiazole	6.0	0.68
Imidazole	0.75	0.83
1,2,4,-Triazole	0.90	0.56
Benzothiophene	0.375	0.56
Indole	0.36	0.63
Benzoxazole	0.19	0.67
Benzothiadiazole	0.23	0.64
Benzimidazole	0.19	0.48
Benzotriazole	0.75	0.15
Dibenzofuran	1.5	0.01
Dibenzothiophene	1.5	0.00
Carbazole	1.5	0.42
Check	——	0.57

insoluble chemotherapeutants) and dried in an oven at 80°F. for 24 hours.

RESULTS.—Five-membered unsubstituted heterocycles were not chemotherapeutic. Thus, thiophene, pyrrole, thiazole, imidazole, and 1,2,4-triazole did not reduce disease symptoms in plants infected with *F. oxysporum* f. *lycopersici* (Table 1). Compounds resulting from the condensation of these heterocycles with 1 benzene ring failed with but 2 exceptions, benzothiazole (4) and benzotriazole, to reduce disease symptoms. Benzothiophene, indole, benzimidazole, benzoxazole, and benzothiadiazole were ineffective as chemotherapeutants (Table 1). However, thiophene and furan condensed with 2 benzene rings to form dibenzothiophene and dibenzofuran, respectively, were active as chemotherapeutants (Table 1). Fluorene, the aromatic hydrocarbon analogue of dibenzothiophene and dibenzofuran, was likewise chemotherapeutic (Table 4). In contrast, carbazole, the nitrogen analogue, was without significant activity (Table 1). Foliage application of dibenzofuran, dibenzothiophene, and fluorene were not chemotherapeutic.

Opening the carbon to carbon bond between the 2 benzene nuclei generally resulted in a loss of chemotherapeutic activity. Diphenyl ether, diphenyl sulfide, and diphenylmethane did not reduce disease significantly. Only diphenylamine produced a chemotherapeutic effect (Table 2).

Six-membered unsubstituted heterocycles were not chemotherapeutic whether or not condensed with ben-

TABLE 2.—*Chemotherapeutic activity of diphenyl compounds in the Fusarium wilt assay*

Compound	Concentration x10^{-3} M	Chemotherapeutic Activity Vasular Index
Diphenyl ether	0.09	0.52
Diphenyl sulfide	0.08	0.77
Diphenylmethane	0.72	0.37
Diphenylamine	0.09	0.09
Check	——	0.61

TABLE 3.—*Chemotherapeutic activity of 6-membered unsubstituted heterocycles in the Fusarium wilt assay*

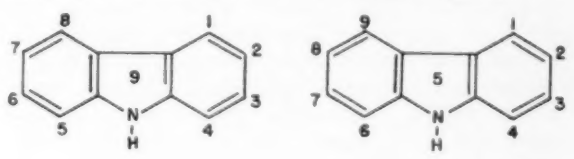
Compound	Concentration x10^{-3} M	Chemotherapeutic Activity Vascular Index
Pyridine	3	0.67
sym-Trithiane	1.5	0.73
Quinoline	0.135	0.65
Isoquinoline	0.09	0.31
Acridine	0.04	0.48
Benzo [f] quinoline	0.006	0.51
Phenazine	0.01	0.76
Thianthrene	0.75	0.53
Phenothiazine	0.09	0.52
Phenoxathiin	0.18	0.54
Check	——	0.57

zene. With the possible exception of isoquinoline, none of the materials listed (Table 3) reduced disease.

The anomalous behaviour of carbazole in contrast to the striking chemotherapeutic potency of dibenzofuran, dibenzothiophene, and fluorene was considered worthy of further investigation. Carbazole differs from its oxygen, sulfur, and carbon analogues in one important respect; the melting point of carbazole is considerably higher than its analogues (Table 4). The high melting point is indicative of association of molecules through hydrogen bonding. Molecular aggregation could result in a structural unit which is too large for penetration of a cell membrane. To test the hypothesis that molecular aggregation is responsible for the inactivity of carbazole, a N-methylated derivative, 5-methylcarbazole[4] was assayed for chemotherapeutic activity. Methylation prevented hydrogen bonding, and accordingly reduced the melting point. The N-methylated carbazole behaved as predicted, i.e., it was able to penetrate the host (as indicated by its high phytotoxicity) and produce a marked chemotherapeutic effect at non-phytotoxic levels. Likewise, a cyanoethyl substitution in the 5-position of carbazole reduced the melting point and produced a chemotherapeutic response (Table 4).

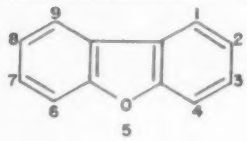
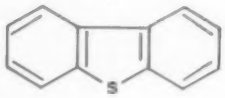
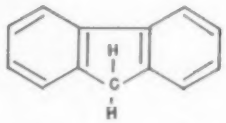
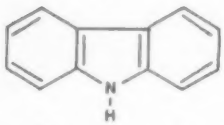
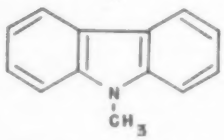
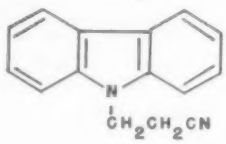
The chemotherapeutically active heterocycles are non-fungitoxic to the parasite in vitro. 5-Cyanoethylcarbazole at higher concentration appeared to stimulate growth. These materials are apparently not chemotherapeutic by virtue of inherent fungitoxic properties (Table 4). Although the chemotherapeutants are relatively nonphytotoxic, they do characteristically cause

[4] According to The Naming and Indexing of Chemical Compounds by Chemical Abstracts (8), carbazole is numbered as shown in I. The alternate number system II is used throughout this article to stress the analogy of carbazole with dibenzofuran and dibenzothiophene.



II

TABLE 4.—*Chemotherapeutic and fungicidal activity of 5-membered heterocycles condensed with 2 molecules of benzene*

Compound	Structure	Melting Point C°	Chemotherapeutic Activity Concentration $\times 10^{-3}$ M	Chemotherapeutic Activity Vascular Index	Fungicidal Activity Concentration p.p.m.	Fungicidal Activity Mycelial weight mg.
Dibenzofuran		87	0.25	0.00	500	53
			0.12	0.04	100	53
			0.06	0.27	10	50
Dibenzothiophene		98	0.12	0.00	500	65
			0.06	0.06	100	67
			0.03	0.53	10	57
Fluorene		116	0.12	0.01	500	73
			0.06	0.04	100	52
			0.03	0.29	10	46
Carbazole		246	2.50	0.30	1000	105
			1.25	0.56	500	90
			0.62	0.34	100	85
			0.31	0.69		
5-Methylcarbazole		87	0.04	0.02	500	94
			0.02	0.07	100	100
			0.01	0.25	10	103
5-Cyanoethylcarbazole		155	1.13	0.02	500	143
			0.56	0.06	100	76
			0.28	0.04	10	70
Check			—	0.79	—	57

defoliation of the tomato host's lowermost leaves. This suggests that the chemotherapeutants do affect the host's biochemical processes.

The chemotherapeutic activity of benzotriazole (Table 1) is probably related to its plant growth regulating properties (3). Benzotriazole breaks apical dominance in tomato plants and causes fasciation of the newly formed leaves. The affected leaves have elongated petioles and a reduced number of leaflets per leaf.

DISCUSSION.—The chemotherapeutic activity of unsubstituted heterocycles is, with but a few exceptions, limited to 5-membered rings condensed with 2 benzene rings. Furthermore, oxygen, sulfur, carbon, and substituted nitrogen are interchangeable in the 5-position without a concurrent loss of chemotherapeutic potency. The equivalent biological activity is not unexpected if the similarity in electron arrangement of the interchangeable atoms is considered. First, oxygen, sulfur, carbon, and nitrogen have their valencies arranged in a tetrahedral geometry. Second, oxygen, sulfur, and the hydrides of carbon and nitrogen, $-CH_2-$ and $-NH-$, respectively, have the same number of covalent bonds. Friedman (6) has grouped O, S, $-CH_2-$, and $-NH-$ into a class of bio-isosteres based on the fact that they have the same number of covalent bonds and are interchanged in many biological reactions. Burtner and Lehmann (1) have in fact shown that the dibenzofuran and dibenzothiophene analogues of the anesthetic β-diethylaminoethyl carbazole-2-carboxylate, produce a similar biological response in rat cornea.

In evaluating the mode of action of fungicides, Rich and Horsfall (8) have divided certain fungitoxic molecules into toxic and lipoid moieties. They have shown that the expression of fungitoxicity is influenced by the nature of the lipoid substituent on the toxiphore. Conceivably, the 5-membered rings in dibenzofuran and its analogues may be the toxiphores whereas the 2 benzene nuclei may merely impart the proper lipoid balance for cell permeation. However, the evidence suggests that this mechanism is not of primary importance. The chemotherapeutic potency of furan and thiophene was not enhanced by lipoid soluble alkyl substituents.

The contrasting chemotherapeutic potency of carba-

zole and of N-substituted carbazoles may be due to the cell permeating qualities of the molecules as influenced by their differences in molecular volume. The role of molecular volume in cell permeation has been established (7). Carbazole, as indicated by its high melting point, probably occurs in molecular aggregates due to hydrogen bonding. In contrast, the lower melting point N-substituted carbazoles presumably do not form molecular aggregates. They may produce a chemotherapeutic effect because the structural units are small enough to permeate the cell membrane. Accordingly, the physical aspects of the compound can be of paramount importance.

Two lines of evidence are available for interpreting the mechanism of action of dibenzofuran and its analogues. First, neither dibenzofuran, dibenzothiophene, fluorene, nor N-substituted carbazole are fungitoxic to *F. oxysporum* f. *lycopersici* in vitro, second, dibenzofuran and its analogues do effect the host's biochemical process, as manifested by chlorosis and eventual loss of the lowermost leaves. Non-fungitoxic compounds with growth regulating effects do induce resistance to Fusarium wilt of tomato (3). The evidence obtained from these earlier investigations indicated that the plant growth regulators produced a chemotherapeutic response by altering the metabolism of the host in a direction unfavorable for disease development. Since dibenzofuran and its analogues do resemble the plant growth regulators as chemotherapeutants, in that they are poor fungitoxicants and partially modify the host's physiology, it is tentatively hypothesized that they reduce disease by producing changes in the host's biochemical process rather than by direct fungitoxic action.

Department of Plant Pathology and Botany
The Connecticut Agricultural Experiment Station
New Haven 4, Connecticut

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EFFECT OF THE STING NEMATODE ON EXPRESSION OF FUSARIUM WILT IN COTTON[1]

Q. L. Holdeman and T. W. Graham[2]

SUMMARY

Greenhouse tests were initiated using the sting nematode and the cotton fusarium wilt fungus. Both fusarium resistant and susceptible cotton varieties were planted in the following treatments: 1) soil with sting nematode, 2) soil with sting nematode plus fusarium, 3) soil with fusarium, and 4) control soil free of sting nematode and fusarium. The resistant cotton succumbed to wilt only when the sting nematode and fusarium were present in combination. The susceptible variety showed a greater percentage of wilt than the resistant variety. With fusarium alone, there was no wilt on the resistant variety and a very low percentage of wilt on the susceptible variety.

Numerous statements in the literature indicate that root knot nematodes, *Meloidogyne* spp., and the meadow nematode, *Pratylenchus* sp. influence the severity of the fusarium wilts of cotton and other crops, and also of other soil-borne fungus diseases. Such statements are based on observation (2,3,10,17), on breeding work (11,14), and on results from soil fumigation (8,12,13,15,16). Direct evidence of the importance of nematodes as a factor in the severity of fusarium wilt of cotton has not been presented.

The early laboratory work is not conclusive. Arndt and Christie (1) concluded that the addition of *Aphlenchoides parietinus, Aphelenchus avenae, Cepha-*

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[2] Respectively, Assoc. Plant Pathologist, S.C. Agr. Exp. Sta. and Pathologist, Field Crops Research Branch, A.R.S., U.S.D.A., Pee Dee Exp. Sta., Florence, S. C.

lobus elongatus, and *Acrobeles butschlii* to cultures did not influence the amount of cotton wilt in greenhouse trials. McClellan and Christie (7), in studying root knot nematodes and fusarium wilts of gladiolus, tomato, and squash, concluded that root knot nematodes had very little effect on the incidence of infection by fusarium. Recently, Sasser, et al, (9), have shown that root knot nematodes affect the expression of black shank on tobacco.

As fusarium wilt of cotton became widely distributed and resistant cottons were widely grown, varieties were found to be resistant in some locations but not in others. Wilds[3] attributed some of the increased susceptibility of resistant varieties to damage caused by root knot nematodes. However, an explanation was lacking for the occurrence of wilt in a field where "there was no evidence of nematode." Based on the behavior of certain varieties at 3 different locations in South Carolina, he postulated 3 different pathogenic races of the cotton wilt fusarium which he designated as Hartsville, Sumter, and Manning wilts. Although this postulation seemed to be logical at the time, supporting evidence has not been presented.

The authors found the sting nematodes in 1 of the fields formerly used by Wilds on which he postulated the race of "Manning wilt." It is suggested that the situation Wilds encountered was not a response of cotton varieties to races of the cotton wilt fusarium but a response to a fusarium wilt-nematode complex involving different nematodes or combinations of nematodes.

Prior to 1951, there was no evidence that ectoparasitic nematodes were involved in the fusarium wilt-nematode relationship. Preliminary experiments by the authors (6) demonstrated that the resistant cotton, Coker 100 WR, was susceptible to wilt when the sting nematode was present. Since other parasitic nematodes were present in these experiments, further work was necessary to determine whether the sting nematode alone was capable of affecting the severity of fusarium wilt in the presence of fusarium.

MATERIALS AND METHODS. — Sting nematodes (*Belonolaimus gracilis* Steiner, 1949) were removed from infested field soil by Christie and Perry's technique (4). To establish soil cultures, the sting nematodes were picked up singly and transferred to a drop of water on a hollow slide for confirmation of their identity. Twenty nematodes were collected in each drop of water, then were washed from the slide and poured around oat and soybean seeds planted in pots of autoclaved soil. Several cultures were established using 100 nematodes per culture. Non-infested pots of autoclaved soil were also seeded with oat and soybean seeds. Both lots of soil were handled in the same manner throughout. As nematode populations increased, each soil culture was handled and increased separately by mixing infested soil with additional autoclaved soil and reseeding[4].

When a sufficient amount of nematode-infested soil was obtained, the 2 lots of soil, nematode infested and non-nematode infested, were subdivided and repotted. Half of each lot of soil was infested with fusarium (*F. oxysporum* Schlecht. f *vasinfectum* (Atk.) Snyder & Hansen), as described below, giving the following treatments: 1) soil with sting nematode, 2) soil with sting nematode plus fusarium, 3) soil with fusarium, and 4) control soil free of sting nematode and fusarium.

Eight 6-inch pots in each of these treatments were seeded as follows: 4 pots to the resistant cotton variety, Coker 100 WR, and 4 pots to the susceptible variety, Hurley's Rowden, after which they were transferred to a greenhouse.

Separate tests were initiated on different days, January 16, 23, and 24, 1953, using different sources of the cotton wilt fusarium to prepare inoculum. For Test 1, 3 mass isolates, isolated from wilted Coker 100 WR cotton collected from 3 separate sting nematode infested fields, were grown for 3 days in liquid media[5]. Immediately before seeding, 100 ml. of the mixed inoculum from 3 flasks was added at a level 1 in. below the soil surface to each pot in treatments 2 and 3. For Test 2, a pathogenic isolate[6] was grown on sterilized wheat seeds for 1 week. The pots were infested by adding a teaspoon of inoculum and seeded as in Test 1. For Test 3, a second isolate was grown on liquid media and handled as described for Test 1. The nematode population in each pot was checked at the beginning and the end of each experiment.

After the cotton seedlings emerged, they were thinned to 10 plants per pot. Records of the number of plants showing external symptoms of wilt were taken. The experiments were terminated 70 days after seeding.

RESULTS.—The data from the 3 tests are in Table 1. Where fusarium was used as inoculum, the percentage of wilted plants of the resistant variety was zero throughout, even though the fusarium isolates used were isolated from wilted plants of the resistant variety. Furthermore, an examination of the stem of each plant revealed no internal symptoms. With the susceptible variety, the percentage of plants showing wilt symptoms was 12.5, 0.0, and 2.5, respectively, for the 3 tests. Where the combination sting nematode-fusarium inoculum was used, the percentage of wilted plants was 80.0, 41.6, and 57.5 for the resistant variety, and 87.5, 52.5, and 95.0 for the susceptible variety.

When soil with the sting nematode alone was used, a trace of wilt occurred in only 2 cases: 5.0 per cent of

[3] Wilds, G. J. 1937. Cotton wilt, a national problem. Coker's Seed Catalogue. Spring 1937. Coker's Pedigreed Seed Co., Hartsville, S. C.

[4] Fungi parasitic on the sting nematode had been encountered in earlier attempts to increase single species cultures; therefore, the different cultures were not bulked to form a uniform population before initiating the experiments.

[5] One dried Fleischmann's yeast cake and 1 bouillon cube per liter of water.

[6] Two different isolates, originating from single spores, tested for pathogenicity, were obtained through the courtesy of Dr. G. M. Armstrong, Clemson Agricultural College, Clemson, S. C.

TABLE 1.—*Relation of the sting nematode to the occurrence of cotton wilt in greenhouse cultures*

Variety and Treatment	Percentage of plants showing external symptoms of wilt[a] Test 1	Test 2	Test 3
Coker 100 WR			
1. Nematode	0.0	0.0	2.5
2. Nematode + fusarium	80.0	41.6[b]	57.5
3. Fusarium	0.0	0.0	0.0
4. Control	0.0	0.0	0.0
Hurley's Rowden			
1. Nematode	5.0	0.0	0.0
2. Nematode + fusarium	87.5	52.5	95.0
3. Fusarium	12.5	0.0	2.5
4. Control	0.0	0.0	0.0

[a] Final readings, 70 days after seeding.
[b] In 2 pots the plant stand was 7 and 9 plants.

the plants of the susceptible variety in Test 1, and 2.5 per cent of the resistant variety in Test 3. The former was probably due to contamination by splashing water, the latter to seed-borne infection. Plants in the control showed no symptoms of wilt.

DISCUSSION AND CONCLUSIONS.—The sting nematode causes severe damage to cotton. It inhibits the development of root tips and causes root decay (5, Fig. 1). In the authors' preliminary experiments (6), other parasitic nematodes were present throughout and caused considerable damage to roots in pots which were infested with Fusarium but were free of the sting nematode; nevertheless, the resistant cotton showed no evidence of fusarium wilt. The exact way in which the sting nematode affects resistance has not been determined. The experience in the preliminary experiments would indicate that for resistant cottons, it is not simply the damaging of the cortex of the root and affording a point of entry for the pathogenic fungus. The results of the present work clearly indicate that the sting nematode, *Belonolaimus gracilis*, greatly facilitates the development of fusarium wilt in both susceptible and resistant cotton plants.

SOUTH CAROLINA AGRICULTURAL EXPERIMENT STATION
FLORENCE, SOUTH CAROLINA

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ISOLATES OF CHAETOMIUM THAT PROTECT OATS FROM HELMINTHOSPORIUM VICTORIAE[1]

Martin Tveit[2] and M. B. Moore[3]

SUMMARY

Chaetomium isolated from oat seeds produced in Brazil, S. A., was antagonistic to various genera of fungi and bacteria in agar culture. The Chaetomium also protected seedlings of oats grown from seed, either naturally or artificially infected with *Helminthosporium victoriae*, from damage by this parasite. This protective effect was more pronounced at low and moderate temperatures than at high temperatures, but was evidenced at all temperatures and under all conditions tested, including nonsterile soil. Both *H. victoriae* and Chaetomium survived for more than 3 months in soil that was alternately moist and dry and in which several generations of oat seedlings were grown, and the protective effect of Chaetomium persisted throughout this time. This suggests that once certain strains of Chaetomium are established in the soil they may continue to protect oat seedlings from infection by *H. victoriae* for a considerable length of time, under a variety of environmental conditions, and in the presence of a variety of other microflora.

The pathogenicity of some soil fungi is influenced greatly by associated microflora (1, 2, 4, 6, 7). Organic or green manures have been used to alter microbiological balance in the soil and to reduce loss from cotton root rot (5). *Bacillus subtilis* has completely protected barley plants from *Helminthosporium sativum* in steamed soil and has greatly reduced infection in field soil (1). Presumably competition for essential nutrients, antibiosis, and parasitism all play a part in determining relationships among microorganisms in soil and disease hazards for a crop plant. The present work deals with the antagonistic effects of Chaetomium strains, or their products, on root-rot organisms.

MATERIALS AND METHODS.—*Source and identity of the Chaetomiums.*—In more or less routine isolations of fungi from oat seed produced in Brazil, S. A.,[4] Chaetomium was obtained frequently. Some of the cultures of Chaetomium strongly inhibited other fungi, such as Helminthosporium and Fusarium, growing from nearby seeds on the same plate. A number of cultures of Chaetomium were isolated and separated into 3 general groups, A, B, and C, on the basis of cultural characteristics. Dr. J. Walton Grovers, Senior Mycologist, Canadian Department of Agriculture, identified type B as *C. globosum* Kze, and type C as *C. cochliodes* Palliser. The species to which type A belonged could not be determined because type A soon lost ability to fruit in culture.

Tests of antagonism in agar plates.—Isolates of Chaetomium were grown at room temperature on nonacid potato-dextrose agar in Petri plates. When the cultures covered more than ½ the diameter of the plates, plugs, 5 mm. in diameter, of agar and fungus were removed and placed on test plates in which the agar had been inoculated at several locations with fungi, or had been seeded with bacteria that were added to the cooled agar before the plates were poured. The criterion of relative amount of inhibition was the width of the clear zone around the plug of Chaetomium.

Tests with seedlings in soil.—Inoculum of *Helminthosporium victoriae* and of Chaetomium was increased by growing the fungi for 5–10 days in potato-dextrose broth in Erlenmeyer flasks. One hundred ml. of medium were used per 250-ml. flask, and the cultures incubated at room temperature. Just prior to application of this to the soil, 400 ml. of sterile water were added to the 100 ml. of broth and fungus from each flask and this mixture was comminuted in a Waring blendor. One hundred ml. of each fungus suspension (total of 200 ml.) were added to the soil in each pot, at the level at which the seeds were to be planted, the seeds were added, and covered with 1 in. of soil. Four-in. pots were used, and 30 seeds were planted in each of 2 pots. Controls were treated in the same way except that the suspension of the inoculum was autoclaved before use.

When the tests involved sterile soil, the soil and pots were autoclaved just before the tests were made. The soil of the greenhouse bench was drenched with a 1:300 solution of formaldehyde, covered with heavy paper, and the pots set on this paper.

Unless otherwise stated, the seed used was a high grade lot of Vicland oats free of *Helminthosporium victoriae* and, so far as could be determined by isolations on agar and by emergence tests in the greenhouse, free of other fungus parasites also. To determine the effect of *H. victoriae* on the stand, the soil in the pots was inoculated with this fungus alone; to determine the effect of Chaetomium on the pathogenicity of *H. victoriae*, the soil in the pots was inoculated with both fungi at the same time. Non-inoculated controls were used in all tests unless otherwise specified. Additional details of procedure are given where the specific tests are described.

RESULTS.—*Inhibition by Chaetomium of bacteria and fungi in culture.*—Typical inhibition of *Helminthosporium pedicillatum* by Chaetomium is shown in Figure 1. Of the various fungi tested for sensitivity to

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Paper No. 3156—Scientific Journal Series, Minnesota Agricultural Exp. Station.

[2] Formerly Research Assistant in Plant Pathology, Dept. of Plant Pathology and Botany, University of Minnesota.

[3] Instructor of Plant Pathology, Dept. of Plant Pathology and Botany, University of Minnesota.

[4] Seed sent by Mr. José A. de Mattos, Parada Experimental, Bage, Rio Grande do Sul.

FIG. 1. Antibiotic effect of Chaetomium (3 colonies near the periphery) upon Helminthosporium pedicellatum.

the antibiotic produced by Chaetomium, the most sensitive appeared to be species of Helminthosporium, Fusarium, Alternaria, and Rhizoctonia. Mucor and Trichoderma were not inhibited until actually in contact with the mycelium of Chaetomium. Chaetomium proved to be strongly antagonistic to all the bacteria tested. These included *Erwinia carotovora*, the cause of soft rot of potatoes and other vegetables, and several unidentified but different kinds of bacteria isolated from plant parts and miscellaneous materials.

Tests in soil.—Autoclaved soil in the greenhouse was inoculated with each of 2 races of *Helminthosporium victoriae*, and with these plus Chaetomium. Vicland oats free of pathogenic fungi were then planted in the soil, and the pots kept at about 23°C. Typical results are in Tables 1 and 2. In these tests, Chaetomium almost completely prevented damage by *H. victoriae*.

Tests similar to the above were made, in which the pots, after the oat seeds were planted in them, were kept at 12°, 23° and 30°C. *H. victoriae* is known to be more pathogenic at higher temperatures than at lower temperatures within this range (1). The results are in Table 3.

In this test Chaetomium inhibited the pathogenicity of *H. victoriae* almost completely at 18°C., and reduced it greatly at 23°C., and at 30°C. Where Chaetomium was present, as many seedlings emerged (56 and 57 out of 60 seeds) as in the controls (55 out of 60 seeds), whereas in those inoculated with *H. victoriae*, alone, the emergence averaged 2.5 seedlings from 60 seeds. At the 3–4 leaf stage, at 30°C., the pots inoculated with *H. victoriae* plus Chaetomium had an average of 38 healthy seedlings, the controls 55, and those inoculated with *H. victoriae* alone had none. Thus the protective effect of Chaetomium was high

TABLE 1.—*The results of inoculating Vicland oats in autoclaved soil in the greenhouse with races H 67 and 1 of Helminthosporium victoriae, with and without the addition of Chaetomium*

Inoculum			Condition of the plants in 3–4 leaf stage		
H. victoriae	Chaetomium[a]	Emergence from 60 seeds	Number dead	Number with blighted mesocotyl	Number healthy
H 67	None	0	–	–	–
R 1	None	13	9	4	0
H 67	Type B	56	4	5	47
R 1	Type C	57	0	4	53
None	None	57	0	0	57

[a] Type B grows faster than type C and is somewhat more antagonistic.

and relatively constant throughout, but was somewhat less at the higher temperatures than at the lower.

Tests with seed naturally infected with H. victoriae.—Seeds naturally and heavily infected with *H. victoriae* were inoculated with Chaetomium. The inoculum was prepared by removing perithecia and mycelium from agar cultures of Chaetomium, and grinding this in a mortar. This inoculum was dusted on the seeds or suspended in water in which the seeds were subsequently shaken. The seeds were then planted in nonsterile soil in pots in the greenhouse, at 23° and 30°C. Thus if "natural" infection responded to the antagonistic effect of Chaetomium, differently from the artificial infection described in the previous section, or if the antagonistic effect were modified greatly by other components of the soil microflora, this test could be expected to reveal it. Results are in Table 4. Even with seed naturally infected by *H. victoriae*, and planted in nonsterile soil, where other organisms abound, the protective effect exerted by Chaetomium was virtually as

TABLE 2.—*The antagonistic effect of Chaetomium spp. on H. victoriae as measured by the development of seedling blight in Vicland oats*

Inoculum			Condition of the plants in 3–4 leaf stage		
H. victoriae	Chaetomium	Emergence from 60 seeds	Number dead	Number with blighted mesocotyl	Number healthy
H 67	None	4	4	0	0
R 1	None	9	5	4	0
R 2	None	21	16	5	0
R 3	None	34	10	19	5
H 67	A[a]	57	0	0	57
R 1	A	59	0	0	59
R 2	A	58	0	0	58
R 3	A	57	0	0	57
H 67	B[a]	54	3	4	47
R 1	B	57	0	2	55
R 2	B	57	0	1	56
R 3	B	56	0	0	56
None	A	58	0	0	58
None	B	58	0	0	58
None	None	58	0	5	53

[a] Type A has a faster growth than type B and is slightly more antagonistic.

TABLE 3.—*The influence of soil temperature on the antagonistic effect of Chaetomium sp. on H. victoriae, in autoclaved soil in the greenhouse*

Soil temperature	Inoculum		Emergence from 60 seeds	Condition of the plants in 3–4 leaf stage		
	H. victoriae	Chatomium		Number dead	Number with blighted mesocotyl	Number healthy
30°C.	H 67	None	4	4	0	0
	R 1	None	1	0	1	0
	H 67	A	57	14	7	36
	R 1	A	56	10	6	40
	None	None	55	0	0	55
23°C.	H 67	None	14	8	3	3
	R 1	None	7	4	3	0
	H 67	A	57	8	10	39
	R 1	A	56	6	8	42
	None	None	56	0	0	56
18°C.	H 67	None	6	4	2	0
	R 1	None	23	10	2	11
	H 67	A	58	0	3	55
	R 1	A	56	0	0	56
	None	None	56	0	0	56

great as in the tests involving artificial inoculation of autoclaved soil. Apparently Chaetomium can survive, grow, and produce its antagonistic effect under a considerable range of conditions.

Survival of H. victoriae and Chaetomium in the soil.—Mixtures of races 1 and H 67 of *H. victoriae*, alone and in combination with Chaetomium, were inoculated into soil in pots as described above. For each inoculation, 30 seeds of Vicland oats free of disease were planted in each of 2 pots, and grown in a greenhouse at about 23°C. for 25 days. The soil was then allowed to dry out and remain dry. The seedlings, of course, died. Seeds of Vicland oats free of *H. victoriae* were planted in some of these pots after 7 days, in others after the soil had dried out for 42 days, and in still others 2 successive crops of seedlings were grown, the soil then permitted to dry out for an additional 40 days (a total of 107 days from the time inoculum was added to the soil) and seeds of Vicland oats free of infection by pathogenic fungi were again planted.

TABLE 4.—*Effect of Chaetomium spp., applied by 2 methods, on disease development in Vicland oats grown from seed naturally infected with H. victoriae*

Soil temperature	Chaetomium, type and condition	Emergence from 60 seeds	Condition of the plants in 3–4 leaf stage		
			Number dead	Number with blighted mesocotyl	Number healthy
30°C.	A Dry[a]	59	0	5	54
	B	54	0	0	54
	C	56	0	1	55
30°C.	A Wet[b]	55	7	0	48
	B	58	2	7	49
	C	54	3	1	50
Control	–	59	13	33	13
23°C.	A Dry	56	0	9	47
	B	58	0	4	54
	C	54	0	3	51
23°C.	A Wet	57	6	3	48
	B	59	4	8	47
	C	56	2	4	50
Control	–	57	11	27	19

[a] Ground culture treatment.
[b] Spore suspension treatment.

In all cases in which the soil was inoculated with *H. victoriae* alone, a high percentage of the plants died, including those planted 107 days after the soil was inoculated (100 per cent of the seedlings were killed or diseased in this latest planting). In all cases where Chaetomium had been added to the soil at the beginning of the test, including the test 107 days after the soil was originally inoculated, a high percentage of the seedlings emerged and a high percentage of them remained healthy. Thus both *H. victoriae* and Chaetomium survived in this soil for more than 3 months and through several successive periods of moistening and drying, and through several generations of seedlings. Likewise, Chaetomium continued to exert its protective effect apparently undiminished throughout the period of the test.

Test with flax.—A single test was made with flax seed. Kommedahl and Christensen (3) have shown that mechanically injured flax seed planted in nonsterile soil usually yields poor stands, presumably because the seed is rapidly invaded by various fungi. Seeds of Crystal variety flax with severe mechanical injuries were inoculated with Chaetomium and planted in nonsterile soil in the greenhouse, and kept at about 30°C. Twenty control seeds yielded only 2 seedlings, while the 20 seeds inoculated with Chaetomium produced 12 healthy seedlings.

By itself this test was too small to be of much significance, but the results agree so closely with those obtained in the more extensive tests with oats that they are at least of interest. Apparently the protective effect of Chaetomium is not limited to *H. victoriae* on oats.

DISCUSSION AND CONCLUSIONS.—Both *Helmintho-*

sporium victoriae and Chaetomium spp. were isolated commonly from oat seed grown in Brazil. *H. victoriae*, however, does not commonly cause any appreciable damage to oats in Brazil, although strains of *H. victoriae* isolated from the oats from Brazil and inoculated into varieties of oats known to be susceptible to this fungus, caused typical Victoria blight in the greenhouse at St. Paul. The lack of any important Victoria blight in Brazil might therefore be attributed, at least in part, to the natural presence on oat seed or in the soil in Brazil of antagonistic species, strains, or varieties of Chaetomium in sufficient amounts to protect the germinating seeds and seedlings from infection.

Whether a fungus such as Chaetomium could be adapted to practical use in protecting oats or other plants from pathogenic fungi was not, of course, proven by these tests. The data suggest rather strongly, however, that antagonisms of this or related kinds might well have practical applications. Certainly such phenomena deserve more intensive study from various standpoints.

Department of Plant Pathology and Botany
University of Minnesota
Institute of Agriculture
St. Paul 1, Minnesota

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COLONIZATION OF BANANA ROOTS BY FUSARIUM OXYSPORUM F. CUBENSE AND OTHER SOIL FUNGI [1]

R. H. Stover and B. H. Waite

SUMMARY

Quantitatively or qualitatively, the grinding or Waring blendor method of preparing colonized banana roots for culturing to determine fungus species present was superior to the commonly used direct plating method. *F. oxysporum* f. *cubense* is a weak saprophytic colonizer of buried Gros Michel banana roots, and *F. solani* and *Cephalosporium* spp. were the dominant saprophytic colonizers, followed later by *Aspergillus* spp. There were no consistently significant differences in degree of colonization or length of survival in colonized roots in soils treated with aqueous solutions containing 400 p.p.m. of $ZnSO_4$, $LiCl_2$, $MnSO_4$, and borax and soils to which these chemicals were not added. *F. oxysporum* f. *cubense* was recovered from less than 15 per cent of the roots from field diseased plants. The stele of the rhizome and not the root system was the main center of fungus activity following infection and few roots were invaded even in advanced stages of disease. *F. oxysporum* f. *cubense* did not readily invade healthy banana roots in the laboratory and was not consistently recovered from rhizosphere soil of diseased plants in the field.

Little is known about the saprophytic phase of *Fusarium oxysporum f. cubense* (E.F.S.) Sny. & Hans., the organism causing Panama disease or banana wilt. It is known that certain infested soils free of bananas and abandoned for 40 years still harbor the fungus in sufficient quantity to preclude growing therein a susceptible variety such as Gros Michel. This may be explained partly by evidence (12) that the fungus colonizes certain grass roots and stems.

An established method of determining the saprophytic potentialities of a root disease fungus is to measure its ability to colonize detached, buried host tissue in competition with the soil micro-flora. This has been done by removing host tissue from infested soil after various periods of time, washing, surface sterilizing, and plating directly on acidified plates in potato-dextrose agar (5, 6, 10, 13). Fusaria studied by this method were *F. culmorum* (5), *F. vasinfectum* (10), and *F. udum* (6). These species were classified as soil inhabitants rather than root inhabitants (3) because they possessed sufficient saprophytic potentialities to compete with the general micro-flora in colonizing detached parts of their respective hosts. Stover and Waite (9) described an improved method for iso-

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TABLE 1. *Number of colonies of fungi developing from banana roots cultured directly, and blended and diluted previous to culturing*

Roots from Sample No.	1		2		3		4		5		6		
	D[a]	M	D	M	D	M	D	M	D	M	D	M1	M2
No. pieces plated	22	—	13	—	20	—	18	—	23	—	29	—	—
Gm. root macerated	—	1.95	—	.85	—	1.66	—	5.20	—	3.93	—	3.25	10.70
F. oxysporum f. *cubense*	6	261	2	178	0	95	1	185	4	49	0	.9	4
F. solani	29	33	14	50	50	53	22	0	25	25	11	1	0
Cephalosporium[b]	0	200	0	200	0	200	0	2000	0	2000	0	80	0
Other fungi	0	12	1	8	1	16	5	9	5	13	1	6	0

[a] D = total number of colonies developing from roots plated directly.
M = total number of colonies in thousands per gram of blended roots.
M1 and M2 = blended black, non-functioning and blended white, functioning roots, respectively, from Panama-diseased plant.

[b] *Cephalosporium* spp. consisted of slow growing pin point colonies usually so numerous that only an estimation of the numbers of colonies was made.

lating fusaria from plant tissue which also gave better measurements of root colonization (8). This new method has been applied to root colonization studies with the banana wilt *Fusarium*. The results reported in this paper indicate that variations in past published studies may have been due to lack of sufficient disintegration of tissue samples for culturing.

GENERAL METHODS.—Segments of white, firm Gros Michel banana roots ½ in. thick and 2 in. long were dispersed throughout a tumbler of soil artificially infested with *F. oxysporum* f. *cubense*. The soils used and method of infesting have been described in a previous publication (7). After this treatment, the root segments were removed, washed in tap water, submerged in 1:1000 $HgCL_2$ for 1½ minutes and washed again. A root sample, usually 1–5 gm., was weighed, ground in a mortar with pestle, and added to 99 ml. of sterile water or, with larger samples, the roots and water were blended in a Waring blendor. The resulting suspension of root fragments was shaken and portions (0.25 to 1 ml.) were transferred to plates of peptone dextrose agar (9). If preliminary tests showed that fungus or bacterial growth on the culture plate was too dense, a further dilution of the suspension was made. Plate counts of colonies were expressed as fungi per gm. of blended root.

To study the effect of method of plating on determination of colonizing species, root segments were removed from glass containers of soil infested with *F. oxysporum* f. *cubense* and from a Panama-diseased banana plant. Five samples of segments were taken from the glasses, 3 from sterilized soil after 90 days and 2 from non-sterilized soil after 35 days. Samples from the Panama-diseased plant consisted of black, non-functioning roots, and white, functioning roots growing in gravel artificially infested with the *Fusarium* and supplied with essential plant nutrients. Each piece of root from the above noted samples, approximately 2 in. long, was cut in half and one portion was plated directly and the other blended, diluted, and plated.

RESULTS.—*Effect of method of preparation for plating on determination of fungus species in banana roots.*—The results (Table 1) of culturing root samples by plating directly and blending previous to culturing show that the latter method gives a truer picture of colonizing fungi present. When plated directly, every root was surrounded by a dense growth of bacteria and the more rapidly growing *F. solani* inhibited or obscured the development of *F. oxysporum* f. *cubense*. Also, certain species of slow-growing fungi such as *Cephalosporium* are evident only when the blending method is used.

Saprophytic colonization of detached banana roots by F. oxysporum f. cubense and other soil fungi.—The combined results of 4 colonization experiments are shown in Table 2. Generally, *F. oxysporum* f. *cubense* was a weak invader of detached healthy banana roots held in tumblers of soil unless sterilized soil was used. However, preliminary experiments not shown indicate that if the quantities of inocula used were doubled or tripled the amount of colonization in non-sterile soil was increased accordingly. The predominant and primary colonizers in most soils were *F. solani* and *Cephalosporium* spp., followed later by *Aspergillus* spp. of which *A. fumigatus* was most common. *F. solani* was more prevalent in some soils than in others and appeared to be most active in roots in loam and wet clay loam. If the quantities of inocula were increased, colonization of banana roots by *F. oxysporum* f. *cubense* could be obtained in the presence of *F. solani*.

The reported effect of trace elements on the colonization of host tissue by *Fusarium* spp. (6) suggested exploratory experiments to determine what role such elements might play in the colonization of banana roots. Also, in order to compare any inhibitory effect of these elements with that of a fungicide, nabam was included in these tests. Aqueous applications of 400 p.p.m. of $ZnSO_4$, $MnSO_4$, $LiCl_2$, and borax to loam soil did not influence significantly the length of survival of *F. oxysporum* f. *cubense* or *F. solani* in detached, colonized banana roots maintained in tumblers of soil. When $ZnSO_4$, $LiCl_2$, $NaNO_3$, borax, and nabam (19 per cent active ingredient) at rates of 0.5 and 1 gm. were applied per 235 gm. of dry loam soil increased in weight by the addition of water to 30 per cent saturation, only borax and nabam reduced the *Fusarium*

TABLE 2. *Colonization of detached buried banana roots by F. oxysporum f. cubense and F. solani in sterile and non-sterile loam*

	Non-sterile				Sterile			
	Infested Loam[a]		Loam		Infested Loam		Loam	
Days	F.O.C.[b]	F.S.	F.O.C.	F.S.	F.O.C.	F.S.	F.O.C.	F.S.
11	10	400	0	400	106	0	0	10
13	25	854	0	550	15	20	0	20
40	4	37	0	81	118	0	0	25
66	0	1500	0	1200	50	100	0	15

[a] Soil artificially infested with *F. oxysporum* f. *cubense* and moisture content adjusted to approximately 25 per cent saturation.

[b] F.O.C. = *F. oxysporum* f. *cubense*, F.S. = *F. solani* expressed in hundreds of colonies per gm. of blended roots.

population in soil and colonization of banana roots. These rates of application are equivalent to approximately ½ and 1 ton per acre. Lower rates did not result in significant differences among treatments in degree of colonization or survival in the invaded roots.

Prevalence of F. oxysporum f. cubense in roots and soil from diseased banana plants.—Twelve to 15 functioning banana roots 6–12 in. long were removed from different locations around each of 15 different plants, 2, 4, and 6 weeks after the first symptoms of Panama disease appeared. Samples from each of the groups of roots were blended and plated to determine populations of *F. oxysporum* f. *cubense*. If the samples from a group showed the presence of *Fusarium*, each individual root in the group was plated to determine percentage of roots infected. Less than 15 per cent of the roots tested yielded *F. oxysporum* f. *cubense* although the rhizome or head in each plant was prominently discolored and heavily infected. Even some of the roots showing vascular discoloration failed to yield the fungus. Roots taken from plants in early and advanced stages of disease growing in gravel culture and heavily inoculated with *F. oxysporum* f. *cubense* did not contain the fungus in any abundance. Apparently, once infection occurred, the rhizome and the rhizome stele in particular became the main center of fungus activity, rather than the roots. Platings of black, non-functioning roots from diseased field plants consistently yielded little or no *F. oxysporum* f. *cubense* but an abundance of *F. solani* and *Cephalosporium* with few other fungus species.

Since there was such a paucity of the fungus in roots from diseased field plants, an examination of rhizosphere was made to determine number of *F. oxysporum* f *cubense* present.

Clay loam rhizosphere soil with a pH of 7.4 was plated at a dilution of 1:500 on peptone-dextrose agar with rose bengal and streptomycin added (6). From samples examined, fusaria belonging to the group Elegans were encountered in only 4 samples and then in low numbers. Even when taken from plants in advanced stages of disease, rhizosphere soil yielded few colonies of Elegans type fusaria. However, *F. solani* was encountered in all samples in abundance and *F. moniliforme*, *F. roseum* and *F. episphaeria* were present in most samples. *Aspergillus* spp. and *Penicillium* spp. were predominant components of the fungus flora of the rhizosphere soil.

Root inoculation experiments.—A problem encountered in studying Panama disease was the lack of a rapid and reliable means of testing the pathogenicity of *F. oxysporum* isolates from soil, water, and plant material. Since final identification of pathogenic forms in the section Elegans is dependent upon production of disease in the host, several attempts to develop a test for pathogenicity were initiated.

Healthy banana roots growing from rhizomes were maintained in moisture chambers and inoculated with bits of agar containing a known virulent culture of *F. oxysporum* f. *cubense*. A slight purplish discoloration of the epidermal cells was occasionally noted after several days but no fungus penetration of root tissue was observed. Small banana rhizomes set in damp vermiculite and held for several weeks until good root growth had been obtained were dug up, submerged in Difco nutrient broth cultures of the fungus and carefully replanted. After 2 weeks, discoloration in the steles of some roots became evident and direct platings on agar, after surface sterilization, usually resulted in the *Fusarium* growing from cut ends of the root sections. However, roots showing vascular discoloration did not always yield the fungus. Vascular discoloration and the presence of the fungus was often associated with the less vigorous, unhealthy appearing roots.

Banana pseudostems, when inoculated with cultures of *F. oxysporum* f. *cubense* and other *Fusarium* species, exhibited some degree of discoloration of the vascular strands around the area of inoculation. Inoculations were accomplished by boring a hole in the stem and packing with a corn-meal-sand culture of the fungus. Apparently some *Fusarium* species and forms other than *F. oxysporum* f. *cubense* can create localized vascular discolorations under such conditions. Nevertheless, fungus penetration into vascular elements was at most only slight.

DISCUSSION.—Saprophytic colonization of detached plant tissue and its measurement may be influenced by the soil population of the colonizing species, soil microflora, nutritional and physical state of soil (pH, texture, moisture), type and condition of invaded material, and techniques used in determining colonization. Walker (13) showed that different types of surface sterilization can determine species of fungi

developing on culture plates and Butler (2) considered the importance of soil population concentration. Sadasivan (5) stated that fungi developing from colonized wheat straw may merely be those most easily able to develop into colonies under the particular conditions employed, and may not be those dominant in the actual decomposition of straw. Also Sadasivan (6) and his students found that trace elements influenced colonization and survival of *F. udum* in gram roots whereas in these studies with *F. oxysporum* f. *cubense* in banana roots such influence was lacking or evident only at phytotoxic concentrations. Studies reported in this paper show how different methods of isolation can determine quantitatively and qualitatively the fungus flora appearing on culture plates. By using a grinding or Waring blendor technique, an entirely different picture of root colonization was obtained than when isolations were made directly from the tissue. These different results could be attributed to different materials and techniques used in measuring colonization and survival.

Despite obvious advantages of the blending method, it too suffers from limitations. Variation among samples in numbers of colonies isolated, even the variation among roots of the same treatment, was often great, thus requiring large numerical differences for significance. The influence of surface sterilization, method and extent of blending, sampling and dilution on colony counts is not wholly understood and requires further detailed study.

F. oxysporum f. *cubense* was a weak colonizer of detached banana roots and thus fits Reinking and Manns (4) original classification of this fungus as a soil invader rather than a soil inhabitant. Garrett (3) clarified and expanded this classification into root inhabiting and soil inhabiting fungi. The former are characterized by an expanding parasitic phase on the living host, and by a declining saprophytic phase in its absence. Such behavior was characteristic of *F. oxysporum* f. *cubense* but not *F. solani*, which was a dominant colonizer of detached banana roots and survived where *F. oxysporum* f. *cubense* could not. Preliminary experiments indicate that even when pieces of heavily infected rhizome tissue are placed in soil, they are rapidly invaded by *F. solani* and other saprophytes. Numbers of *F. oxysporum* f. *cubense* in such tissue gradually decline until they are recovered with difficulty. Decline is more rapid in moist soils as contrasted with dry soils. In a previous paper (7) it was stated that *F. oxysporum* f. *cubense* survived in banana roots in submerged soil but was eradicated in surrounding soil. It is now known that this fungus surviving in banana roots was *F. solani*, which was wrongly identified as *F. oxysporum* f. *cubense*.

The presence of such few members of *F. oxysporum* f. *cubense* in the roots of diseased plants even in advanced stages of disease is unique when compared with most other *Fusarium* wilt diseases. This indicates a pronounced resistance of roots to fungus invasion. Wardlaw (14, 15) noted that root infection was hindered by the secretion of wound gum, collapse of vessels, and formation of tyloses, and that invasion occurred through the root bases and not directly into the rhizome. Apparently such mechanisms of resistance operate to a limited extent in the stele of the rhizome since fungus penetration of this tissue is extensive once it is reached. Pathogenicity tests revealed the resistance of healthy roots to infection and are in agreement with Wardlaw's (14) results. Wellman (16) also noted that it is often difficult to find root infection in *F. oxysporum* f. *cubense* infested soil. Roots actually growing through decayed, infected banana heads remained healthy (16).

The resistance of roots to invasion and the paucity of *F. oxysporum* in the rhizosphere of diseased plants indicate a comparatively low rate of disease spread in banana plantations. Thus Volk (11) has listed the "life" of different virgin banana soils with respect to Panama disease. For example, virgin soils with a pH of 6.6 and above, in general, had a production life of 20 years whereas more acid soils (as low as pH 5.6) reduced the productive life of the land. However, he further stated that while the pH of banana soils are of considerable importance they are only indicators of the presence of other unknown factor(s) responsible for spreading disease (11). In most crops attacked by *Fusarium* spp. a life of 10–20 years would be indicative of host resistance. Butler (1) has also shown that resistance to invasion is pronounced. Of 4,593 diseased mats he found that 75 per cent produced marketable fruit subsequent to exhibiting Panama disease symptoms. The total percentage of infected mats producing fruit subsequent to show of symptoms gradually decreased, indicating that volume of fruit production in a diseased area is related to the total density of *F. oxysporum* f. *cubense* present in the soil. As this density increases, the natural resistance of more plants is overcome to the extent that no more fruit is produced (1). Apparently a large amount of inoculum is required for rapid disease spread thus increasing chances of root fungus contact and of some of the invasions breaking through to the stele. Or in other words, the numerical threshold of infection and of disease is relatively high.

Division of Tropical Research
Research Department
United Fruit Company
La Lima, Honduras, C. A.

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THE RELATIONSHIP OF PATHOGENICITY IN PSEUDOMONAS SOLANACEARUM TO COLONY APPEARANCE ON A TETRAZOLIUM MEDIUM[1]

Arthur Kelman

SUMMARY

Mutants of *Pseudomonas solanacearum* differing in colony morphology from the normal or wild type were detected readily when bacterial suspensions from stock cultures were streaked on a medium containing tetrazolium chloride and examined with obliquely transmitted light. The most common mutant formed a round, butyrous, deep red colony with a narrow bluish border. In contrast, the normal or wild type formed an irregularly-round, fluidal, white colony with a pink center. Inoculation tests on tomato seedlings demonstrated that colony appearance on the tetrazolium medium could be related to pathogenicity. Cultures derived from butyrous red colonies were either weakly pathogenic or non-pathogenic, whereas cultures from fluidal white colonies with pink centers were highly pathogenic.

In connection with a study of colony morphology in isolates of *Pseudomonas solanacearum* E.F.S., causal agent of southern bacterial wilt, 3 main colony types were observed. Colonies of the normal or wild type, which characterize fresh isolates obtained from diseased plants, were irregularly-round, fluidal, and opaque. In contrast, the mutant type most frequently encountered in stock cultures formed uniformly round, butyrous, and translucent colonies. In both types the colony surface was smooth. A third type that formed butyrous translucent colonies with a rough surface was observed infrequently. These colony types were similar to certain of those described by Okabe (6, 7) in isolates of *P. solanacearum* obtained in Formosa and Japan.

Although the main colony type in a given isolate could be determined by examination of streaked plate cultures with a dissecting microscope, detection of small numbers of variants in the wild type culture was often difficult. In order to facilitate a study of variation in *P. solanacearum* it was apparent that a medium was needed that would aid in the differentiation of colony types. Tetrazolium chloride had been used in solid media for detection of certain biochemical mutants in *Escherichia coli* (4) and for detection of morphological variants in *Pasteurella pestis* (5) and certain other bacteria. The investigations reported in this paper were initiated to determine 1) whether differences among colony types would be accentuated on a medium containing tetrazolium and 2) whether pathogenicity to tomato of a given colony type could be correlated with its appearance on a tetrazolium medium. A preliminary report on certain phases of these studies has been presented (2).

EXPERIMENTAL METHODS.—Dilute bacterial suspensions were streaked on a medium of the following composition: 1.0 per cent peptone, 0.1 per cent casein hydrolysate (Difco), 0.5 per cent glucose, 1.7 per cent agar, and 0.005 per cent triphenyl tetrazolium chloride in 1 liter of distilled water. Proper aliquots of a sterile 1 per cent aqueous solution of tetrazolium were added

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aseptically to the melted medium to obtain the desired concentration prior to pouring the plates. The streaked plates were incubated at 32°C. for 36 hours and then examined under a dissecting microscope with obliquely transmitted light, following the procedure described by Henry (1).

Bacterial suspensions were prepared from stock cultures which had been stored under sterile mineral oil for periods ranging from 6½ years to 6 months. The method used in preparing these cultures for storage has been reported previously (3). The cultures used represented isolates from diseased potato, tobacco, tomato, peanut and pepper plants collected in different areas in North Carolina. Tomato plants of either the Bonny Best or the Rutgers variety were inoculated by placing a drop of bacterial suspension at the third node below the stem tip and forcing a needle into the stem through the drop. At the time of inoculation plants were usually 14-16 in. in height with 6-7 expanded leaves. Disease readings based on a numerical rating scale previously described (6) were taken at 7 day intervals.

Single cell isolations were made with the Chambers micromanipulator. Growth of single cells was obtained in a sterile medium of the following composition: 1.0 per cent tryptone, and 0.5 per cent glucose in 1 liter of distilled water.

EXPERIMENTAL RESULTS.—In all cultures, marked differences in the intensity of red coloration were evident between colonies of wild and variant types. Colonies of the wild type were either entirely white or white with a light red area in the center of the colony, whereas the colonies of mutant types were deep red with a very narrow light bluish border. These differences were most apparent when plates were inverted and the undersides of colonies examined. In cultures containing small mutant populations the colonies of mutants could be detected as tiny red circular spots, even when completely surrounded by the coalescing fluidal white growth of the wild type. Differences in color intensity between colonies decreased after 72 hours.

In order to confirm the assumption that the atypical colony types found in cultures were variants of *P. solanacearum*, single cells were isolated from a fresh culture of the wild type obtained from an inoculated tomato plant. When streaked on the tetrazolium medium, the cultures of single cell origin initially formed only the typical fluidal white colonies with pink centers. However, after 3-5 days of growth in 1 per cent tryptone broth, mutants could be detected that formed non-fluidal red colonies identical in appearance on the tetrazolium medium to those formed by the mutants observed in stock cultures.

Tests were then initiated to compare the virulence of cultures derived from mutant and wild type colonies. In repeated inoculations of tomato plants in greenhouse experiments, cultures derived from single mutant colonies that showed a dark red pigmentation on the tetrazolium medium were either weakly virulent or avirulent. In contrast, cultures from wild type colonies which remained relatively free of coloration during the initial 36 hours of growth were consistently highly pathogenic.

The reliability of colony appearance on the tetrazolium medium as a criterion of virulence was further demonstrated in the following experiment. This test was initiated in order to determine whether virulent cultures could be obtained consistently by colony selection from isolates containing mixtures of virulent and weakly virulent or avirulent types. Dilute bacterial suspensions of the isolates listed in Table 1 were made from stock cultures preserved under mineral oil. When these suspensions were streaked on 1 day-old plates of the tetrazolium medium, it was apparent that colony types were present to a varying degree in all stock cultures. One well-isolated single colony of the wild type was selected from each of 17 different isolates; a single colony of the mutant type was selected from each of 5 isolates. These single colonies were suspended in sterile water blanks and streaked on the tetrazolium medium. The procedure of re-streaking a single colony from each of the 22 cultures was repeated 5 times at 72 hour intervals. Single colonies selected from the fifth series of streaked plates were re-streaked on a nutrient medium of the following composition: 1.0 per cent tryptone, 0.1 per cent yeast extract, 0.5 per cent glucose, and 1.7 per cent agar in 1 liter of distilled water. At the end of 72 hours, 1 loop of bacteria was removed from each of these cultures and suspended in 2 ml. of sterile tap water. Eight Rutgers tomato plants were inoculated for each isolate.

Results are summarized in Table 1. All cultures considered to be pathogenic on the basis of the characteristic appearance of colonies on the tetrazolium medium brought about very rapid wilting of inoculated plants. At the end of 14 days, all plants inoculated with these virulent cultures were either severely wilted or dead. In marked contrast, none of the mutant type cultures had produced pronounced wilting symptoms or plant death 21 days after inoculation. However, stunting, vascular discoloration, and adventitious root formation appeared in plants inoculated with the mutant type cultures of isolates 5 and 16.

The tetrazolium medium has also been evaluated as an aid in detecting the wild colony type in isolations from diseased tobacco, tomato, potato, and eggplant. In those instances in which bacterial suspensions were obtained from severely diseased plants and streaked on the tetrazolium medium, many different kinds of colonies often appeared, including those of various saprophytic bacteria. Because of the distinctive appearance of the wild type colonies of *P. solanacearum* on this medium, however, selection of these typical white colonies from mixed cultures was greatly facilitated. Single colonies selected in this manner were re-streaked until examination with the dissecting microscope indicated that the cultures were free of other bacteria. All isolates obtained by this procedure were highly pathogenic to tomato.

TABLE 1.—*Pathogenicity to tomato of cultures of Pseudomonas solanacearum classified on basis of colony appearance on the tetrazolium medium*

Cultures Tested			Disease Index[a]	
Number	Host from which isolated	Number of months stored under sterile mineral oil	Colony appearance Fluidal-white	Non-fluidal-red
3	Potato	78	85	
4	Potato	78	98	
5[b]	Potato	66	—	20
9	Potato	30	100	
51	Potato	6	90	0
16	Tobacco	66	95	20
17	Tobacco	66	100	
18	Tobacco	66	88	
40	Tobacco	42	100	
41	Tobacco	42	98	
45	Tobacco	42	100	
46	Tobacco	30	100	
47	Tobacco	30	100	
48[b]	Tobacco	6	—	0
25	Tomato	42	100	
60	Tomato	3	100	0
60-R[c]	Tomato	0	100	
31	Peanut	66	100	
80	Pepper	6	93	

[a] Disease index 14 days after inoculation.
[b] Wild type colony was not found in stock culture.
[c] 60-R was a fresh re-isolate of 60 following passage through a moderately resistant tobacco plant (Dixie Bright 101).

DISCUSSION.—Isolates of *P. solanacearum* often lose virulence rapidly when maintained in culture by routine methods. The procedure of storing cultures under sterile mineral oil has been found to aid in maintaining isolates in a pathogenic state for 4 years (3). In these studies it was found that storage under mineral oil did not prevent the development of mutants of reduced virulence; however, the virulent wild type had not been completely eliminated in most instances. Furthermore, pathogenic cultures could be recovered from isolates stored for as long as 6½ years under mineral oil.

Evidence was also obtained that loss in virulence of cultures could be attributed to mutation and selection that resulted in populations dominated by weakly virulent types, differing in colony morphology from the wild form. This conclusion confirms in part results obtained by Okabe (7) in his studies on the pathogenicity of colony types of *P. solanacearum* in Japan. In macroscopic observations of morphological characteristics of colonies, Okabe noted that weakly pathogenic or avirulent mutants differed in colony morphology from the fluidal wild type. However, he also reported in tests on tomato and tobacco that all fluidal colonies were not necessarily highly pathogenic. Moreover, virulence was variable among morphologically similar colonies derived from a single virulent fluidal colony. This latter finding differs from results obtained thus far in pathogenicity tests on tomato with isolates from North Carolina. All cultures have been highly pathogenic that were derived from wild type colonies selected on the basis of their characteristic appearance on the tetrazolium medium.

The application of these results has facilitated the initiation of a more detailed study of the relationship between colony morphology and virulence of *P. solanacearum*.

PLANT PATHOLOGY DEPARTMENT
NORTH CAROLINA STATE COLLEGE
RALEIGH, NORTH CAROLINA

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SYMPTOMS IN THE FLORISTS' HYDRANGEA CAUSED BY TOMATO RINGSPOT VIRUS AND AN UNIDENTIFIED SAP-TRANSMISSIBLE VIRUS[1]

Philip Brierley[2]

SUMMARY

Four collections of hydrangeas affected with tomato ringspot were obtained from Maryland, North Carolina, and Pennsylvania. Infected plants were unsalable, but incidence of ringspot was generally low. Symptoms varied, but the most characteristic was dull-yellow chlorosis of leaves with diffuse green blotches superimposed. Infected plants were dwarfed, leaves irregular in shape, and flowers small and pale.

Tomato ringspot virus was readily isolated from infected hydrangeas. The virus was identified by cross-protection with Price's type strain and by its close agreement with the type strain in symptoms produced in 17 test species including tobacco, tomato, and hydrangea. The following new hosts of tomato ringspot virus are reported: *Antirrhinum majus* L., *Datura stramonium* L., *Eschscholtzia californica* Cham., *Gilia capitata* Dougl., *Helichrysum bracteatum* Andr., *Hydrangea macrophylla* (Thunb.) DC., *Petunia hybrida* Vilm., *Senecio cruentus* DC., *Silene compacta* Fisch., *Spinacia oleracea* L., and *Tropaeolum majus* L. No infection was produced in *Pelargonium hortorum* Bailey or *Reseda odorata* L., plants previously reported susceptible.

Characteristic symptoms of tomato ringspot were produced in hydrangea seedlings by inoculation with sap from infected hydrangeas and by inoculation with sap from tobacco infected with tomato ringspot virus, including Price's strain. Typical symptoms developed 8 months after graft inoculation and 14–20 months after sap inoculation.

Chlorotic rings, brown rings, and vein patterns common in hydrangea are caused by an unidentified virus, or viruses, sap-transmissible to hydrangea seedlings but not infectious to tobacco. No significant injury has been associated with such symptoms thus far.

In earlier papers (1, 2) tomato ringspot virus was reported associated with dieback and mosaic in hydrangeas (*Hydrangea macrophylla* (Thunb.) DC.) received from commercial producers in Pennsylvania and North Carolina, and the presence of a second virus in some of the source plants was suggested. We can now define the characteristic symptoms of tomato ringspot in hydrangea and can distinguish these from the symptoms produced by an additional virus, or viruses, much more common in this host but less damaging to it than the tomato ringspot virus.

MATERIALS AND METHODS.—The various collections of diseased hydrangeas serving as virus sources are described under "Symptoms". Seedling clons of hydrangea were supplied in May, 1952, by Conrad B. Link, of the University of Maryland, as leaf bud cuttings made in 1952 from seedlings derived from his crosses of 1950. Originally there were 100 plants representing 59 seedling clons. Some clons were discarded, and others were increased and used in our tests in 1952 and later. All hydrangeas were grown in pots in a greenhouse throughout the year, except for 6 weeks' storage at 40°F. during November and December, 1952, and January and February, 1954.

Of the other plants inoculated, only geranium (*Pelargonium hortorum* Bailey var. *Rosina*) was propagated by cuttings. All others were grown from seed and inoculated during the early stages of active growth. Inoculations were made chiefly by wiping crude expressed juices usually prepared in M/10 Na_2HPO_4, over leaves previously dusted with carborundum powder. Yarwood's (7) method of inoculation with fresh leaf tissue also proved useful in inoculations from hydrangea to tobacco. A few inarch grafts were made between diseased hydrangea plants and hydrangea seedlings.

SYMPTOMS.—The symptoms of tomato ringspot are extremely varied both in naturally infected and in experimentally infected hydrangeas. Only 4 collections of hydrangea affected with this disease have come to our attention. A shoot dieback, causing considerable commercial loss in a Pennsylvania greenhouse in 1947, yielded the tomato ringspot virus. No other pathogen was detected. Samples of this collection have been maintained by vegetative propagation for 7 years, but dieback symptoms have not recurred and the cause of dieback is still uncertain. Symptoms in these plants now correspond closely to those expressed by the North Carolina Strafford plants reported in 1952 as having rugose mosaic (1). The most characteristic symptom in both lots is a dull-yellow chlorosis of leaves with diffuse-bordered dark-green blotches superimposed (Fig. 1.B). Plants are dwarfed; leaves are smaller than normal, often irregular in shape, frequently narrow, occasionally but not typically rugose, and sometimes stiff and brittle. Flowers are generally smaller than normal; they tend to open irregularly and have green and colored flowers in the same cyme; the color of the sepals is more dilute than normal. Hydrangeas from a second Pennsylvania greenhouse in 1954 had narrow leaves with irregular margins and a poorly defined gray etch; sepals were small and pale pink. A plant of Engel's White hydrangea from N. W. Stuart's forcing tests at Beltsville in 1953 expressed a mild gray surface etch in leaves in April 1954, and yielded the tomato ringspot virus. None of the diseased plants submitted by commercial

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[2] Senior Pathologist, Section of Ornamental Plants, Horticultural Crops Research Branch, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland.

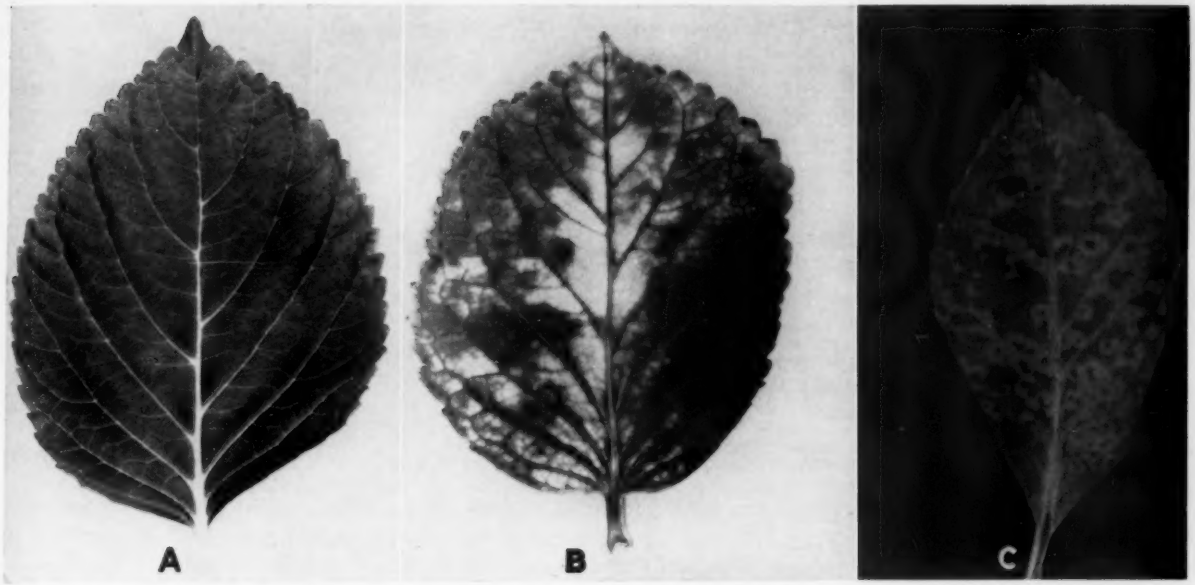

FIG. 1. A) Normal leaf of hydrangea seedling; B) Leaf of hydrangea seedling showing typical symptoms of tomato ringspot; C) Ringspots in a leaf of Strafford hydrangea caused by a sap-transmissible virus not infectious to tobacco.

growers expressed the characteristic symptoms of tomato ringspot illustrated in Fig. 1,B when received. Evidently growers noticed the earlier symptoms of leaf and flower abnormalities.

Symptoms caused by some virus other than the tomato ringspot virus are common in hydrangeas. Specimens from Ohio in January 1949, and from New Jersey in May, 1951, showed yellow blotching and at times chlorotic rings in leaves. Hydrangeas from commercial sources forced at Beltsville by N. W. Stuart frequently showed chlorotic blotches, chlorotic rings (Fig. 1,C), brownish rings, or oakleaf patterns in the older lower leaves; less commonly they expressed yellowish vein patterns in young leaves. Plants expressing these symptoms force satisfactorily. It is not certain that a single virus causes chlorotic rings, brown rings, oakleaf patterns and vein patterns, but the tomato ringspot virus from tobacco does not produce these symptoms in hydrangea seedlings. Since these symptoms are common in hydrangea they are likely to accompany tomato ringspot infections. For example, the Pennsylvania specimen of 1954 yielded tomato ringspot virus in March, and expressed conspicuous brown ringspot after flowering in April. As shown in Table 1 a sap-transmissible virus producing chlorotic rings and yellow veining, as well as the tomato ringspot virus, was found in the Strafford hydrangeas from North Carolina. Also, ring and oakleaf patterns developed in leaves of 2 of 59 seedling clons furnished for this study in 1952. Development of these symptoms in 2 seedling clons only suggests the possibility that a seed-borne virus may be involved.

DETECTION AND IDENTIFICATION OF TOMATO RINGSPOT VIRUS. — Tomato ringspot virus was detected in hydrangea on subinoculation to tobacco at all seasons of the year when hydrangeas were growing actively. The number of spots on 3 tobacco plants ranged from 6 to 413, with no correlation whatever between number of spots and intensity of symptoms in the source plant.

Tobacco plants recovered from the hydrangea virus symptoms were not protected against tobacco ringspot or cucumber mosaic virus. Zinnia plants recovered from symptoms of the hydrangea virus were not protected against the indicator strain of cucumber mosaic virus. Identity of the hydrangea virus with the tomato ringspot virus (5, 6), *Annulus zonatus* Holmes (4), was suspected from similarity in host plant reactions and from Hildebrand's (3) finding of this virus in red currant, another member of the Saxifragaceae.

The Price culture and the hydrangea culture were then compared. Rooted cuttings from the tops of tobacco plants recovered from the hydrangea virus were fully protected against the Price culture. The hydrangea virus is therefore considered to be tomato ringspot virus.

When the 2 virus cultures were inoculated into various test species in parallel there was very good agreement in symptom production between the Price virus and the hydrangea virus, but some discrepancies appeared between the reactions found and those previously reported (6) for the tomato ringspot virus. Species marked "(H)" were tested against the hydrangea culture only, and others were inoculated with the Price culture also.

Plants of the following species developed systemic symptoms, and the virus was reisolated: *Antirrhinum majus* L., snapdragon; *Callistephus chinensis* (L.) Nees, China aster (H); *Cucumis sativus* L., cucumber (H); *Helichrysum bracteatum* Andr. (H); *Hydrangea macrophylla* (Thunb.) DC; *Lycopersicon esculentum*

TABLE 1.—*Experimental production of symptoms in hydrangea by inoculation with tomato ringspot virus and an unidentified ringspot virus*

Date and method of inoculation	Source of inoculum	Hydrangea plants inoculated	Plants infected [a]	Symptoms expressed and dates	Reisolation of tomato ringspot virus [b]
		1952			
June 12 (sap)	North Carolina hydrangea var. Strafford, dull, yellow leaves with diffuse green blotches	10 seedlings	6/10	Chlorotic rings, yellow veining (Feb. 2, 1953; Mar. 8, 1954)	0/6
			1/10	Symptomless through March 1954	1/4
June 12 (graft)	North Carolina hydrangea var. Strafford, dull, yellow leaves with diffuse green blotches	2 normal Strafford plants	2/2	Dull-yellow leaves with green blotches (Feb. 2, 1953)	2/2
July 11 (sap)	Tomato ringspot virus from tobacco	5 seedlings	4/5	Dull-yellow leaves with green blotches (Mar. 8, 1954)	4/5
November 5 (sap)	Tomato ringspot virus from tobacco	10 seedlings	0/10	None	0/10
November 18 (sap)	Tomato ringspot virus (Price culture) from tobacco	7 seedlings	1/7	Dull-yellow leaves with green blotches (Mar. 22, 1954)	1/7
		1953			
January 29 (sap)	Tomato ringspot virus from tobacco	5 seedlings	1/5	Pink flowers blotched white (Mar. 23, 1953)	1/1
			5/5	Dull-yellow leaves with green blotches (Mar. 22, 1954)	5/5

[a] Number infected over number inoculated.
[b] Number of plants found infected over number tested.

Mill., tomato; *Nicotiana tabacum* L., tobacco; *Petunia hybrida* Vilm., petunia; *Phlox drummondii* Hook.; *Platycodon grandiflorum* (Jacq.) A. DC.; *Senecio cruentus* DC., cineraria; *Verbesina encelioides* (Cav.) B. & H.; *Zinnia elegans* Jacq.

Plants of the following 5 species expressed no symptoms but the virus was reisolated: *Eschscholtzia californica* Cham., California poppy; *Gilia capitata* Dougl.; *Sinningia speciosa* B. & H., gloxinia (H); *Spinacia oleracea* L., spinach; *Tropaeolum majus* L., nasturtium.

Three species of plants expressed local lesions, but the virus could not be recovered in tobacco on subinoculation: *Datura stramonium* L.; *Tetragonia expansa* Murr., New Zealand spinach; *Silene compacta* Fisch.

The following plants expressed no symptoms, and the virus could not be reisolated from them: *Cheiranthus cheiri* L., wallflower; *Dianthus plumarius* L.; *Pelargonium hortorum* Bailey; *Philadelphus coronarius* L., mock orange (H); *Physalis angulata* L.; *Physostegia virginiana* (L.) Benth.; *Portulaca grandiflora* Hook. (H); *Reseda odorata* L., mignonette.

The following new hosts of the tomato ringspot virus were not recorded by Price (6): snapdragon, helichrysum, hydrangea, petunia, cineraria, verbesina, *Datura stramonium*, and *Silene compacta*. Four additional plants recorded as insusceptible by Price (6)—California poppy, *Gilia*, spinach, and nasturtium—yielded the virus on subinoculation in our trials. On the other hand, Price reported *Pelargonium* and *Reseda* as susceptible, but we failed to infect them and could not recover virus from them. Such discrepancies may be due to testing different varieties of a given species or to variations in the titer of the tomato ringspot virus in the source plants. The very close agreement in performance of the type culture and the hydrangea culture in our tests indicates that the latter is the type strain or differs insignificantly from it.

EXPERIMENTAL PRODUCTION OF SYMPTOMS IN HYDRANGEA.—Hydrangea inoculations in 1952-53 are summarized in Table 1. Inarch grafting diseased Strafford hydrangeas of the North Carolina source to apparently normal Strafford in June, 1952, induced the typical yellowed leaves with green blotches after 8 months. The source plants also expressed these symptoms at that time and subsequently, so that there has been satisfactory reproduction of symptoms by grafting since early 1953. Symptoms were first well defined in February, 1953, about 1 month after the plants were returned to the greenhouse from storage, during a period of rapid growth or forcing. Tomato ringspot virus was detected in both inoculated plants and in both source plants after 7 months and again after 21 months.

Sap inoculation of hydrangea seedlings from the

same Strafford source plants at the same date failed to reproduce the typical tomato ringspot symptoms in 1953 or 1954. Six of 10 plants inoculated expressed chlorotic ring patterns in 1953 and yellow vein patterns in 1954, but these plants failed to yield the tomato ringspot virus on subinoculation after 4, 9 and 21 months. One of the 4 symptomless plants yielded the tomato ringspot virus in parallel tests at each of these 3 periods.

Sap inoculation of hydrangea seedlings with tomato ringspot virus from tobacco in July and November, 1952, and in January, 1953, also failed to induce typical symptoms in 1953, although subinoculations detected the virus in 10 of 27 seedlings inoculated. Thus it seemed likely at the end of 1953 that the tomato ringspot virus was of incidental importance in hydrangea, or possibly of importance only in combination with an unidentified virus, or other viruses, also present in the Strafford source plant. One of 5 seedlings inoculated on January 29, 1953, developed white blotches in pink sepals 2 months later, an uncommon symptom of tomato ringspot; this virus was recovered on subinoculation from the blotched sepals.

When the hydrangeas inoculated in 1952–53 were again forced in 1954, 10 of 11 plants infected with the tomato ringspot virus by sap inoculation expressed characteristic symptoms comparable to the symptoms in the source hydrangeas. One plant which consistently yielded the virus on subinoculation remained symptomless. Duplication of the typical dull yellowing and green blotching by sap inoculation with tomato ringspot virus from tobacco shows that this virus causes these symptoms. At the same time the hydrangea virus that is not infectious to tobacco continued to produce only mild rings or veining symptoms in the second season after infection. Thus it appears that the tomato ringspot virus is damaging to hydrangea and the unidentified hydrangea virus causes little injury.

For a semi-woody plant, hydrangea is readily infected with the tomato ringspot virus by sap inoculation. Tests made in June and July, 1953, when the plants were growing actively, and in January, 1954, when elongation after storage was rapid, yielded 4/5 and 5/5 infections, respectively. Similar tests in November were much less successful, but Price's type strain of tomato ringspot virus did infect 1 plant and induce the characteristic response in hydrangea. In further tests in April, 1954, 4/10 hydrangea seedlings inoculated by sap from the Strafford source hydrangeas, and 9/10 inoculated from a previously infected hydrangea seedling became infected, as shown by subinoculation to tobacco by the Yarwood method 6 weeks later.

U. S. PLANT INDUSTRY STATION
BELTSVILLE, MARYLAND

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MANEB—A COINED NAME FOR THE FUNGICIDAL CHEMICAL MANGANOUS ETHYLENEBIS(DITHIOCARBAMATE)

The word maneb has been selected as a coined name for the fungicidal chemical manganous ethylenebis(dithiocarbamate). This name has been approved by the Interdepartmental Committee on Pest Control. The American Phytopathological Society, the American Chemical Society, and the American Medical Association are agreeable to its use. Approval of the name maneb implies that the name is available for free use in designating the pure chemical in question.

Maneb has previously been known by its chemical name, manganous ethylenebis(dithiocarbamate), and also as managanese EBD, MnEBD, and on occasion, as MEB. The name maneb refers to the pure chemical and a technical grade shall indicate the percentage of maneb present. A wettable powder formulation designated duPont "Manzate" fungicide containing 70 per cent maneb is commercially available. ("Manzate" is a registered duPont trademark.)

ROOT ROT OF ALFALFA CAUSED BY PHYTOPHTHORA CRYPTOGEA[1]

Donald C. Erwin[2]

SUMMARY

This is the first expanded account of Phytophthora root rot of alfalfa. The disease was found in several fields in 5 counties of California.

Symptoms of the disease on alfalfa are yellowing of the leaves, and reddish-brown, irregular necrosis of the periderm and vascular tissues of the tap root. A yellow discoloration is usually present in the tissues above and below the lesion. A *Phytophthora* sp. was isolated from diseased tissue. Mycelium was non-septate, with hyphal swellings developing profusely. Sporangia were non-papillate and ovoid to ellipsoid in shape; oogonia, globose; antheridia, amphigynous; and oospores, globose, measuring less than 30 μ in diameter. Cardinal temperatures for growth were as follows: minimum, 8°; optimum, 25°; maximum, 30°–33°C.

The fungus was identified as *Phytophthora cryptogea* Pethybridge & Lafferty. On no other hosts than some in the genus *Medicago* was it more than slightly pathogenic. Several other isolates of *P. cryptogea*, as well as of other species of *Phytophthora*, were tested on alfalfa, but none except the isolate from alfalfa was pathogenic.

Phytophthora root rot of alfalfa (*Medicago sativa* L.), a previously undescribed disease, was reported recently by the writer (3). The incitant has since been determined to be *Phytophthora cryptogea* Pethybridge & Lafferty. The disease was first noted in June of 1952 in Yolo County, California. Affected areas on tap roots of 6-month-old plants were brown to red in color with a yellow, diffuse discoloration extending above and below the lesions. Later in the year, a survey revealed the presence of the disease in other fields in Yolo County and also in Solano, San Joaquin, and Fresno counties. In 1953, the disease was found on a few plants in a field in Riverside County.

The determination that *P. cryptogea* is an incitant of a root and crown rot of alfalfa explains some of the loss of stand in alfalfa in California. *P. cryptogea* is not, however, the cause of all crown and root rot of alfalfa. Whether or not it contributes to dark crown necrosis, the cause of which has not been determined (4), is not yet known.

The purpose of this paper is to report the symptoms of Phytophthora root rot on alfalfa, to describe the causal fungus, and to present the results of pathogenicity tests on alfalfa and some other plants grown in California.

SYMPTOMS OF THE DISEASE.—Symptoms of the disease on tap roots of alfalfa are illustrated in Fig. 1. The crown, tap roots, or lateral roots of the plant may be affected by the fungus, but the tap root is the part most often attacked. The infected area on the periderm of the root is usually irregular in shape and brown in color, externally. Internally, necrotic areas in newly infected roots are red to brown in color. This tissue may be water-soaked but usually remains firm. Later the affected tissue turns dull yellow to brown, and in severely affected roots the yellowed region may extend 2–3 cm. above and below the lesion. Periderm, phloem, and finally xylem tissues may be affected, and, prior to complete girdling of the roots, the leaves become yellow and flaccid. Ultimately, the plant wilts and dies.

The brown-to-red necrosis in irregular areas and the yellow discoloration of the xylem extending a few cm. above and below the lesion are the distinguishing symptoms of the disease. Yellow and wilting tops are indicative of the disease but are not distinguishing, since these symptoms may be brought about by other causes, such as severing of the tap root by a gopher or severe Fusarium wilt. In an advanced stage of the disease the root symptoms may not be typical, owing to secondary invasion by other microorganisms.

THE CAUSAL FUNGUS.—*Isolation.*—Isolations were made from at least 5 diseased plants of each of 13 collections from 10 fields in Yolo, Solano, San Joaquin, and Fresno counties. In the first isolation, tap roots of 6-month-old infected plants were surface-sterilized with a proprietary sodium hypochlorite solution, "Chlorox" (1:10), and washed twice in sterile water. In later work diseased roots were dipped in 70 per cent ethyl alcohol and blotted dry prior to isolation; this appeared to be the better method. Tissue pieces from the margins of affected areas were plated on potato-dextrose, acid potato-dextrose (pH 4.5), and water agars. On the water agar, a *Phytophthora* appeared on nearly all the tissue pieces, whereas on the potato agars only bacteria developed. All subsequent platings were made on water or corn-meal agars.

In later isolations, the fungus was not easily isolated from plants 1–3 years old. Non-pathogenic forms of *Fusarium roseum*, *F. solani*, and *F. oxysporum* were also isolated frequently. The *Phytophthora* grew from the tissue piece slowly, and, usually, a hyphal tip could not be obtained until 4–5 days after plating. Bacteria often suppressed the fungus.

Pieces of diseased tissue from lesions at various stages of infection were plated on corn-meal agar.

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Paper No. 818, University of California Citrus Experiment Station, Riverside, California.

The initial part of this work was reported in a section of a thesis presented to the Graduate Division, University of California at Davis, in partial fulfillment of the requirements for the degree of Doctor of Philosophy. The work on host range, on some of the temperature studies, and on identification of the fungus was completed at the University of California at Riverside. Appreciation is expressed to Dr. B. R. Houston, Department of Plant Pathology, University of California at Davis, for guidance during the early work.

[2] Assistant Plant Pathologist, University of California Citrus Experiment Station, Riverside, California.

FIG. 1. Symptoms of Phytophthora root rot of alfalfa. A) Longitudinal section of a root naturally infected. B) Irregular brown lesion on bark of a young plant artificially inoculated.

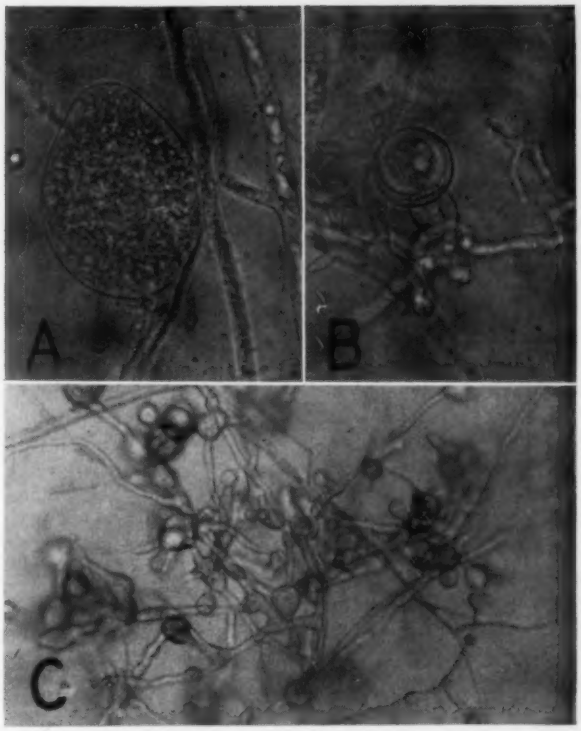

FIG. 2. Morphology of *Phytophthora cryptogea* grown on alfalfa seedlings in sterilized water. A) Non-papillate sporangium (×754). B) Oogonium, oospore, and amphigynous antheridium. Ooogonial stalk can be seen in the antheridium (×754). C) Hyphal swellings (×307).

Isolation of the causal fungus was accomplished most easily from lesions showing the red-brown necrosis typical of early infection. The fungus was never isolated from the yellow tissue above or below the lesion. Care in selecting newly infected tissue is important for isolation of this *Phytophthora* from alfalfa. The difficulty in isolating this slow-growing *Phytophthora* may explain why the fungus has not previously been found to be associated with a root rot of alfalfa.

Morphology.—Young mycelium was non-septate but became septate with age. It grew in a tortuous pattern on water and corn-meal agars. The diameter varied from 7 to 8 μ. On potato-dextrose and water agars, numerous intercalary and terminal hyphal swellings (Fig. 2, C) developed, which were not delimited by septa. They were irregular in shape and often formed in clusters. The contents of the swellings became dense with age and imparted a tan color to older cultures. The swellings were typical of those of *Phytophthora cryptogea* illustrated by Pethybridge and Lafferty (7) and by Blackwell (2).

Sporangia (Fig. 2, A) ranged from 23 to 38 μ in width and from 27 to 63 μ in length, and averaged 31 × 39 μ. They formed sparsely on a sterilized medium consisting of 4 hemp seeds in 15 ml. of water, and on water agar on which alfalfa seeds were allowed to germinate. These bodies usually appeared after 8–12 days. They were nonpapillate and ovoid to obpyriform in shape. Formation of zoospores has not been observed. Irrigation or cooling of colonies growing in water failed to stimulate indirect germination. Germination, at least in vitro, was only by the direct method. Contents of the sporangia were occasionally extruded. Proliferation sometimes occurred in irrigated cultures.

Oogonia (Fig. 2, B) were globose and ranged from 23 to 39 μ in diameter (average, 31 μ). They occurred on the same media as sporangia but much more abundantly and after only 3–5 days. They were also formed sparsely on 4- to 6-week-old cultures of lima bean, tomato juice, and potato-dextrose agars. Oogonia formed abundantly in roots of young alfalfa seedlings which had germinated on agar, and were frequently seen in infected lateral roots from plants artificially inoculated. However, they have not been seen in naturally infested roots. Antheridia were amphigynous (Fig. 2, B). Oospores (Fig. 2, B) ranged from 19 to 37 μ in diameter (average, 25 μ). They were globose and smooth and usually filled the oogonium.

Growth-temperature relations.—The effect of temperature on growth of the fungus was measured by recording the diameter of a colony grown on potato-dextrose agar for 5–6 days and by weighing the oven-dry

(70°C.) mycelium grown on a culture consisting of 50 ml. of a potato-dextrose liquid medium in a 250-ml. Erlenmeyer flask for 6–10 days at different temperatures. Initial inoculum, when the fungus was grown on agar, consisted of a 4-mm. disc of potato-dextrose agar and mycelium from the margin of a growing culture. In some experiments in which a liquid potato-dextrose medium was used, a glass rod was bent to a 90° angle about 15 mm. from one extremity. The bent portion was flattened and served to support a 4-mm. disc of agar and mycelium. A cotton plug was wrapped around the unbent extremity and the bent end inserted into the Erlenmeyer flask. Prior to incubation, the agar disc on the flattened end of the glass rod was lowered to the surface of the liquid medium. In other experiments, 1 ml. of a mycelial suspension, prepared by blending a 5-day-old potato-dextrose-agar plate culture of *Phytophthora* in 50 ml. of sterile distilled water with a Waring blendor, was pipetted into each flask. Basically both methods yielded similar results. However, when initial inoculum was placed on the bent glass rod, dry weights were less variable than when a blended inoculum was used. Blended inoculum sometimes would not grow at 30°C., whereas a 4-mm. disc of agar consistently started at this temperature. In each experiment at least 4 replicates per temperature were used.

The results of 2 typical experiments in which radial growth and mycelial weight were measured are shown in Fig. 3. On potato-dextrose agar no growth occurred at 2°–7°C. and in only a few tests at 8°C. The optimum temperature was 25°C. and the maximum between 30° and 33°C. At 33° the fungus did not always grow, but removal to a lower temperature consistently resulted in growth, which indicated that the fungus was not killed by exposure to 33°C. for 7 days. Growth at 30°C. occurred on corn-meal agar as well as on potato-dextrose agar.

Leach (5) showed that the optimum temperature for growth of *Pythium ultimum* Trow in liquid culture was lower than its optimum temperature on an agar medium. The optimum temperature for growth of *Phytophthora cryptogea* was the same in liquid potato dextrose as on an agar medium (Fig. 3). The minimum temperature for growth in a liquid medium was 8°, optimum 25°, and maximum 30°–33°C. At 33°C., growth occurred only occasionally.

Identification.—The *Phytophthora* sp. that incites root rot of alfalfa resembles both *Phytophthora cryptogea* Pethybridge & Lafferty and *P. cambivora* (Petri) Buis. Tucker found that the cardinal temperatures for *P. cryptogea* were 5°, 25°, and 30°C. Middleton et al (6), working with an isolate from Gloxinia, found them to be 1°, 22°–25°, and 31°–34°C. The cardinal temperatures for the *Phytophthora* from alfalfa were 8°, 25°, and 30°–33°C. Except for a higher minimum these were nearly the same as those for *P. cryptogea*. *P. cambivora* (10) grew at a minimum of 15°, optimum 27°, and maximum, 30°C. Except for the higher minimum temperature for radial growth, the reaction of *P. cambivora* was essentially the same as that of the *Phytophthora* from alfalfa.

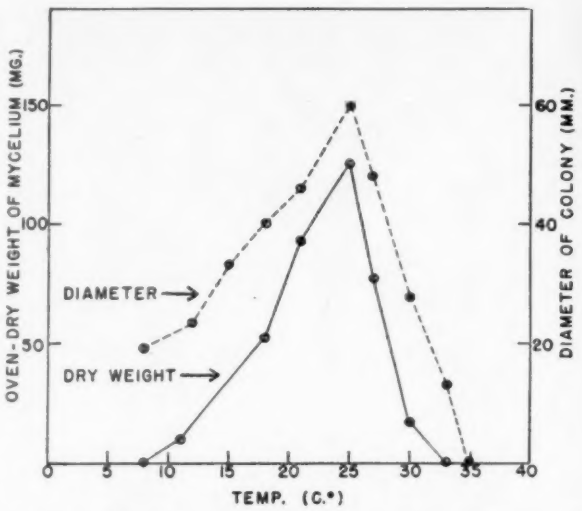

FIG. 3. Effect of several temperatures on the radial growth of *Phytophthora cryptogea* grown on potato-dextrose agar and on the mycelial weight of the fungus grown on a liquid potato-dextrose medium.

One criterion used by Tucker (10) to identify *Phytophthora cryptogea* was the following: "oospores on oatmeal agar, averaging less than 30 μ in diameter." Oospores, although not formed in oatmeal agar by the *Phytophthora* isolated from alfalfa, formed on hemp seed and alfalfa stems and averaged less than 30 μ in diameter. The isolate of *P. cambivora* studied by Tucker did not produce oogonia. Petri (8) reported oospores of *P. cambivora*, in diseased tissue, with a diameter of 20–27 μ, and, later (9), oogonia with a diameter of 57–62 μ. Oospores seen by Ashby (1) in corn-meal agar measured 38.8 μ. In the present work, oospores ranged from 19 to 37 μ in diameter and averaged 25 μ. Although of doubtful taxonomic value, in view of the variance in size reported for *P. cambivora*, the oospores of the isolate from alfalfa more closely resemble those of *P. cryptogea* than those of *P. cambivora*.

Since pathogenicity to apple and potato was used by Tucker (10) as a taxonomic criterion, the *Phytophthora* from alfalfa was placed in wounds in White Rose and Idaho Russett potatoes and in nearly ripe Pippin and Gravenstein apples and incubated in moist chambers at 20°–24°C. for 10 days. On apple, the fungus was only slightly pathogenic and no pathogenicity was noted on potato. The test was repeated several times with similar results. In the pathogenicity reaction to apple and potato the fungus resembled *P. cambivora*, which was reported by Tucker (10) to be only slightly pathogenic to apple and non-pathogenic to potato tubers.

Profuse formation of hyphal swellings (Fig. 3, C) on potato-dextrose agar, on water agar, on sterilized hemp seeds in distilled water, or on alfalfa seedlings germinated in sterilized distilled water was characteristic of the *Phytophthora* from alfalfa. Hyphal swell-

ings of *P. cambivora* were reported and illustrated by Petri (8) and of *P. cryptogea* by Pethybridge and Lafferty (7). Hyphal swellings formed by the alfalfa *Phytophthora* resembled more closely those of *P. cryptogea* illustrated by Pethybridge and Lafferty.

Because of its non-papillate sporangia, oospores less than 30 μ in diameter, growth on corn-meal agar at 30°C., and the similarity of the hyphal swellings to those illustrated for *Phytophthora cryptogea* (2, 7), the fungus on alfalfa is identified as *Phytophthora cryptogea* Pethybridge & Lafferty.

Pathogenicity to alfalfa.—Inoculum for pathogenicity tests consisted of a blended culture of the fungus grown on potato-dextrose agar. This was prepared in various dilutions but was mostly used at the rate of 2 2-week-old plate cultures in 100 ml. of distilled water. Thirty ml. of this inoculum was placed on the top of steamed soil in a 5-in. pot. Later tests showed that much less inoculum was necessary for pathogenesis.

In the first test, 25 Ceresan-treated seeds of California Common alfalfa were planted in an infested 5-in. pot of steamed soil. Emergence was as good in infested soil as in the control soil, but after 30 days all plants in the infested soil had been killed. Seedlings growing in the steamed soil were unharmed. When untreated seeds were planted no emergence occurred in infested soil. In another pot of infested soil, 7 2-month-old plants were transplanted. After 18 days 5 of these plants were wilting and stunted, in comparison with control plants. Tap roots (Fig. 1, B) showed the typical reddish-brown necrosis of diseased plants in the field. This test was repeated, and after 13 days all 42 inoculated plants showed wilting and root rot typical of that occurring on plants in affected fields. None of the control plants was affected. The fungus was reisolated from all the diseased plants. These results have been confirmed by numerous repetitions of the tests.

No controlled-temperature experiments have been conducted, but preliminary observations indicated that the disease progressed less rapidly at greenhouse temperatures of 10°–16°C. than at temperatures of 21°–27°C.

Pathogenicity tests on plants other than alfalfa.—Seeds of other possible hosts were planted in soil infested with a mycelial suspension of the fungus. In most cases blended inoculum was also placed on the roots of plants 1–2 months old. Roots were examined after an incubation period of 1–2 months. As a check on viability of the inoculum, alfalfa was inoculated at the same time. At least 20 plants and an equal number of seeds of each species were inoculated. Plants growing in steamed soil were used as controls. Fourteen species of plants were tested. These included California No. 5 cowpea (*Vigna sinensis* Savi),[3] red kidney bean (*Phaseolus vulgaris* L.), Thomas Laxton pea (*Pisum sativum* L.), purple vetch (*Vicia benghalensis* L.), yellow sweet clover (*Melilotus officinalis* Lam.), red clover (*Trifolium pratense* L.), ladino clover (*Trifolium repens* L.), broadleaf birdsfoot trefoil (*Lotus corniculatus* L.), Imperial flax (*Linum usitatissimum* L.), and Acala cotton (*Gossypium hirsutum* L.). Only large 1- to 2-month-old plants of sugar beet (*Beta vulgaris* L.), Marglobe tomato (*Lycopersicon esculentum* Mill.), aster (*Aster* sp.), and carrot (*Daucus carota* L.) were tested. None of the plants showed any root rot. Cowpea, bean, yellow sweet clover, and red clover showed some lateral root browning, but this was not severe enough to cause the plants to wilt or die. When seed was planted in infested soil, red clover seedlings became infected and died. Roots of 1- to 2-month-old red clover plants were not rotted by the fungus.

It has been reported previously in this paper that the fungus was only slightly pathogenic to apple fruits and non-pathogenic to potato tubers. Fruits of cucumber (*Cucumis sativus* L.), cantaloupe (*Cucumis melo* L.), citron melon (*Citrullus vulgaris* Shrad. var. *citroides*), crook neck squash (*Cucurbita* sp.), tomato (*Lycopersicon esculentum*), orange (*Citrus sinensis* Osbeck), lemon (*Citrus limon* Burm.), and roots of carrot (*Daucus carota*) were inoculated with the fungus and placed in moist chambers for at least 10 days at room temperature (20°–24°C.). Only citron melon and cucumber were affected by the fungus; rotted areas were only 1–1½ in. in diameter after a 9-day incubation.

PATHOGENICITY OF OTHER PHYTOPHTHORA ISOLATES TO ALFALFA.—Since *P. cryptogea* was difficult to isolate from alfalfa, the possibility that other *Phytophthora* spp. might incite a root rot of alfalfa and be missed in routine isolations was considered. Isolates of *Phytophthora cryptogea* from other hosts were also tested.

Three isolates of *Phytophthora cryptogea*, 1 from J. T. Barrett of the University of California at Berkeley, and 2 from the collections of J. T. Middleton and L. J. Klotz of the University of California at Riverside, were tested on alfalfa seedlings, as well as on 1- to 2-month-old plants. Middleton's isolate was a transfer from the type culture from Pethybridge. None of these isolates was pathogenic to alfalfa. In addition, 2 isolates of *Phytophthora parasitica* Dast., 1 from citrus and 1 from alfalfa, *P. cinnamomi* Rands from avocado, *P. drechsleri* Tuck. from safflower, *P. palmivora* Butl. (source unknown), and 4 unidentified Phytophthoras isolated from carrot, sugar beet, sweet clover and alfalfa were tested, but none was pathogenic to seedlings or to plants 1–2 months old.

Since only the isolates of *Phytophthora cryptogea* from alfalfa were pathogenic on alfalfa, and since the work thus far indicates that the host range of the *Phytophthora* from alfalfa may be narrow, there is a possibility that this fungus is a strain that has become adapted to alfalfa and may be specific to the genus *Medicago*.

DISCUSSION.—Although this is the first expanded account of Phytophthora root rot of alfalfa, the disease

[3] Names of plants are those accepted by Bailey, L. H. 1949. Manual of Cultivated Plants. Rev. Ed. 1116 p. MacMillan Co., New York.

probably is not new. Other workers in the past have noted plants with similar symptoms. Failure to recover the inciting fungus has probably been due to difficulty in isolation. The fungus has been obtained by the writer only from newly infected plants, and often not consistently. Although *Fusaria* are consistently isolated from the rotted tissue, workers should be cautious in attributing rots of this type to the *Fusaria*. In California no root rotting *Fusaria* have been found by the writer (4). Phytophthora root rot could contribute to the dark crown necrosis disease, the entire cause of which is not yet known (4). Secondary organisms could suppress *Phytophthora* in the roots during periods that are unfavorable for pathogenesis.

Although *Phytophthora cryptogea* from alfalfa fits the description for this fungus, it apparently differs pathogenically from other *P. cryptogea* isolates tested. The isolate from alfalfa was not pathogenic to tomato and aster, both of which are hosts of *P. cryptogea*. The finding that other isolates of *P. cryptogea* do not attack alfalfa is further evidence of its specificity.

Department of Plant Pathology
University of California
Citrus Experiment Station
Riverside, California

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EFFECTIVENESS OF CERTAIN ANTIBIOTICS FOR THE CONTROL OF SEED-BORNE DISEASES OF SMALL GRAIN[1]

Curt Leben, D C. Arny, and G. W. Keitt

SUMMARY

The antibiotic substances helixins A, B, and C, crystalline antimycin A-35, and crude helixin were tested as seed treatment materials for the control of several diseases of small grain in greenhouse and field experiments. All materials reduced the Victoria seedling blight of Vicland oats. For the control of this disease with helixin B it was necessary for the antibiotic to be carried in ethanol; other carriers, in addition to ethanol, were effective for the control of oat smut and wheat bunt by this antibiotic. Oat smut was also reduced by crude helixin and by antimycin A-35.

In an earlier paper successful greenhouse and field experiments using the antibiotic helixin B as a seed treatment material for the control of several seed-borne diseases of small grain were reported (3). In most of these tests the antibiotic was applied in ethanol, a carrier that possesses several limitations, among which are its expense and its harm to seed under some conditions. In view of these limitations and the fact that helixin B was not effective as a dust for the control of at least 1 disease (3), it was deemed desirable to study the role of ethanol and to see if it could be replaced by other carriers. Experiments toward this end are described in this paper. Also included are accounts of seed treatment tests with other antibiotic materials for the control of small grain diseases. Helixins A and C, antimycin A, and crude helixin were used in these experiments.

Materials and methods.—*Diseases.*—As in the previous study (3), most of the tests were made with the seedling blight of Vicland oats, a disease incited by *Helminthosporium victoriae* M. & M. The Victoria blight was chosen because it could be studied readily in the greenhouse and because previous experience indicated that it was not always controlled by chemicals that were effective for surface borne pathogens (1). The methods of Leben, Arny, and Keitt (3) were used for

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experiments with this disease.

The control of oat smut and wheat bunt was studied in a field test at Madison. The oat variety was Canadian, and the seed was naturally infested with 2 smut organisms, *Ustilago kolleri* Wille and *U. avenae* (Pers.) Rostr. For the test with bunt, seed of wheat variety Sturgeon was artificially infested with chlamydospores of the bunt fungus, *Tilletia foetida* (Wallr.) Liro. In the study of both diseases there were 3 replicates for each treatment, each replicate consisting of 10 gm. of seed in a 3 meter row.

Treatment of seed.—Various types of helixin were used. The material termed crude helixin was the ethanol extract of the precipitate that formed on acidification of the liquid culture filtrate of the helixin-producing *Streptomyces* sp. This material contained 1.3 helixin units per mg. and was 3 manipulations removed from the fermented culture (step 4 of fractionation procedure (4)). Helixins A, B, and C were prepared as noted previously (6). The helixin A sample contained 5 units per mg. Two samples of helixin B were used, preparations 32 and IV-17-B, which contained 2.6 and 3.1 units per mg., respectively. Helixin C was an oily liquid containing 0.01 unit per mg. Also studied was a water soluble preparation containing helixins A, B, C, and D; it was prepared by precipitation of a crude material by NaCl from an ammonium solution and had a potency of 0.8 unit per mg. Details of the preparation of the water soluble material will appear elsewhere.

The antibiotic antimycin A (2) was of the type now designated antimycin A-35 (5). It was in crystalline form.

The concentration of antibiotic preparation used was in part determined on the basis of previous experiments. The aim was to select a level that would not give complete disease control; by so doing some idea of the relative effectiveness of various treatments could be obtained.

In previous studies (3) it was found that if ethanol was used as a carrier for seed treatment materials, seed injury resulted unless the ethanol was allowed to evaporate soon after application. Therefore, a standard procedure was used for ethanol as well as for the other carriers: seed was treated with the test material in 125 or 250 ml. Erlenmeyer flasks, stored in the stoppered flasks for 15–30 minutes, and then spread in shallow pans to dry.

In all tests, nontreated seed and seed treated with Ceresan M (7.7 per cent N-(ethylmercuri)–*p*-toluenesulfonanilide) at a rate of 0.5 oz. per bushel were included for comparison purposes. Seed treated with Ceresan M was stored in a closed container for 4 days or longer before planting. Seed that had been treated with ethanol alone was not included in these experiments, since the earlier work had shown that this carrier did not influence the amount of the several diseases. The other carriers used in the present work, when applied alone, likewise did not appear to influence disease development, nor did they have phytotoxic effects. Except as noted none of the treatments was phytotoxic.

Vapor action.—Vapor action was tested by placing helixin B in a closed container with seed in such a way that the seed and the chemical were not in direct contact (1). In the crock there were 7 gm. of seed and 1.25 gm. of dry helixin B (preparation IV-17-B), an amount of antibiotic sufficient to treat 500 gm. of seed (crock full) at the rate of 2.5 mg. per gm. of seed. The agar diffusion assay employing the fungus *Glomerella cingulata* (Stonem.) Spauld. & v. Schrenk was used for the detection of the antibiotic on the seed.

RESULTS.—*Influence of the carrier on disease control.*—Attention was given to ethanol as a carrier for helixin B in a series of studies with the Victoria blight. The influence of the amount of ethanol was shown in 3 greenhouse tests made at different times. Three mg. of preparation 32 was dissolved in 0.06, 0.125, or 0.25 ml. of ethanol, and the solution used to treat 5 gm. of seed. The average number of blighted seedlings with the different amounts of ethanol was 7, 4, and 2 per cent, respectively. The proportion of ethanol in ethanol-water mixtures was also shown to influence disease control in the same 3 tests. The antibiotic was carried in 0.25 ml. of 25, 50, or 100 per cent ethanol: the average disease was 8, 6, and 2 per cent, respectively. These figures may be compared with the nontreated control, which gave an average of 13 per cent disease, and with the Ceresan M treatment, which produced an average of 1 per cent diseased plants.

Two other solvents and a solvent mixture were also used as carriers for helixin B in greenhouse tests with the Victoria blight. Preparation 32 was dissolved in methyl Cellosolve (ethylene glycol mono-methyl ether), ethylene glycol, or the solvent mixture (43 parts of ethylene glycol, 7 parts of Tween 20, and 50 parts of water, by volume) and applied to the seed at the rate of 0.6 mg. in 0.02 ml. of carrier per gm. of seed. This amount of carrier provided good seed coverage. In 3 tests made at different times the methyl Cellosolve and ethylene glycol treatments each resulted in an average of 9 per cent diseased plants, whereas nontreated seed produced 12 per cent, and the Ceresan M treatment gave 2 per cent. In the same tests, treatment with the antibiotic in 0.05 ml. of ethanol per gm. of seed resulted in 3 per cent disease. In another series of 2 tests the mixture produced an average of 11 per cent diseased plants as compared with 17 per cent in plants from nontreated seed. Thus, these carriers do not appear to be so effective as ethanol for the control of this disease.

The influence of ethanol on the control of the Victoria blight was also studied in 2 greenhouse tests with the water soluble preparation carried in water or in ethanol. The antibiotic was used at a rate of 0.6 mg. in 0.05 ml. of the carrier per gm. of seed. Tests were made at different times. The average disease with water as the carrier was 14 per cent, whereas there was 4 per cent disease with ethanol. Nontreated seed produced plants with 12 per cent disease, and there was

TABLE 1.—*The effect of various carriers on the control of oat smut and wheat bunt by seed treatment with helixin preparations. Field test*

Material	Mg. per gm. of seed	Carrier	Per cent disease	
			Oat smut	Wheat bunt
Helixin B	1.25[a]	Ethanol[b]	0.2	0
Helixin B	0.62	Ethanol[b]	0.7	0.7
Helixin B	0.31	Ethanol[b]	6.6	1.0
Helixin B	0.15	Ethanol[b]	14.2	----
Helixin B	1.25	Mixture[c]	0	0
Helixin B	1.25	Ethylene glycol[b]	0[d]	----
Water soluble helixin	1.25	Ethanol[b]	0.4	----
Water soluble helixin	1.25	Water[b]	0.9	----
Ceresan M	[e]	----------	0	0.2
None	----	----------	10.7	6.3

[a] Equivalent to 0.4 unit.
[b] Used at 0.08 ml. per gm. of oats, 0.04 ml. per gm. of wheat.
[c] Mixture of 43 parts ethylene glycol, 7 parts of Tween 20, and 50 parts water, by volume. Used at a rate of 0.04 ml. per gm. of seed.
[d] Treatment reduced germination about 40 per cent.
[e] On the basis of 0.5 oz. per bushel: 0.9 mg. per gm. for oats and 0.5 mg. per gm. for wheat.

1 per cent disease as the result of the Ceresan M treatment. These experiments also demonstrated the importance of ethanol for the control of this disease.

Various carriers for the control of oat smut and wheat bunt were studied in a field test. Both helixin B (preparation IV-17-B) and the water soluble preparation were used. Results are given in Table 1. In contrast to the findings with the Victoria blight, control of these 2 diseases was obtained with carriers other than ethanol. Another field test with bunt of spring wheat, not reported in detail here, was confirmatory—helixin B carried in the mixture provided the same order of control as the antibiotic carried in ethanol.

Helixins A and C, antimycin A, and crude helixin for the control of the Victoria blight and the smut of oats.—Data for the control of the Victoria blight by these materials in 3 greenhouse tests are given in Table 2. Data for helixin B (sample IV-17-B) are also included for comparison.

Antimycin A at a rate of 1.25 mg. and crude helixin at 0.46 mg. per gm. of seed were tested for the control of oat smut in a field test. Both materials were applied in ethanol, antimycin A at a rate of 0.08 ml. and crude helixin at 0.04 ml. per gm. of seed. The test was made at the same time and had for comparison the same nontreated and Ceresan M-treated controls as the experiment indicated in Table 1. Crude helixin reduced the disease to zero, and with antimycin A the amount of disease was 4 per cent.

Vapor action of helixin B.—In previous studies, several preparations of helixin B were shown to have vapor action in microscope slide tests (3). It became of interest, therefore, to see if the antibiotic could be detected on the seed using experimental conditions

TABLE 2.—*Effectiveness of the various helixin preparations and antimycin A as seed treatment materials for the control of the seedling blight of Victland oats. Greenhouse tests*

Material	Mg. per gm. of seed	Helixin, units per gm. of seed	Per cent disease
Helixin A[a] ----------	0.6	0.6	2
Helixin B[a] ----------	0.6	0.4	3
Helixin C[b] ----------	9.9	0.1	6
Antimycin A[a] ----------	2.4	----	3
Crude helixin[a] ----------	0.3	0.4	4
Ceresan M ----------	0.9[c]	----	>1
None ----------	----	----	18

[a] Carried in 0.05 ml. ethanol per gm. of seed.
[b] Used without dilution at 0.05 ml. per gm. of seed.
[c] Equivalent to 0.5 oz. per bu.

under which vapor action was demonstrated for certain of the organic mercury compounds (1). None was found, however.

DISCUSSION.—In the present work several antibiotics have been shown to be effective for the control of the Victoria blight and the smut of oats, and for wheat bunt. The effectiveness of helixin B, previously reported, has been confirmed. Helixins A and C provided control of the Victoria blight and, for this disease, appear to possess about the same potency as helixin B on a unit basis. It was not surprising, therefore, to find that the crude helixin preparation, which contained all the helixin antibiotics, also controlled the disease. It is concluded that the separation of the helixins would not be essential for seed treatment purposes.

In the present work crystalline antimycin A-35 was shown to reduce the incidence of the Victoria blight and the smut of oats. Partly purified antimycin A-102, a closely related material, has been found to be effective for the control of the barley seedling blight incited by *Helminthosporium sativum* P. K. & B., and for the Victoria blight (5).

In the present studies ethanol has been shown to play an important part in the effectiveness of helixin B for the control of the Victoria blight. Recent work with the organic mercury materials has shown that the most effective compounds for this disease are characterized by high vapor action, and that fungicidal vapors penetrate underneath hulls of the kernel (1). This information suggests that the pathogen is borne at least partly within the outer layers of the seed and that its protected position accounts for the lack of effectiveness of materials that do not readily produce penetrating vapors. On this basis, it seems reasonable to assume that ethanol serves to carry helixin B into the outer parts of the seed. Some evidence for this view is afforded in that helixin B in carriers other than ethanol was effective for diseases incited by the surface borne pathogens of wheat bunt and of oat smut. The specificity of carriers for seed treatment materials has not been demonstrated heretofore to our knowledge.

While it has not been possible to replace ethanol as a carrier of helixin B for the control of the Victoria blight, the solvent mixture offers promise for the control of disease incited by surface borne pathogens. For this type of disease, water soluble helixin carried in water also appears to merit further study.

Department of Plant Pathology
University of Wisconsin
Madison, Wisconsin

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A XANTHOMONAS PARASITIC ON UREDIA OF CEREAL RUSTS[1]

D. S. Pon, C. E. Townsend, G. E. Wessman, C. G. Schmitt and C. H. Kingsolver[2]

SUMMARY

The name *Xanthomonas uredovorus* is proposed for a new species of bacterium parasitic on uredia of cereal rusts. Only rust pustules that have erupted are readily attacked. Symptoms of bacterial infection appeared within 3 days after inoculation of pustules with bacteria when plants were held at 30°C. during the day and at 22°C. during the night. The bacterium caused lysing on stem rusts of wheat, oats and rye, on leaf rusts of wheat and rye, and on crown rust of oats. Uredia of *Puccinia graminis tritici* Eriks. and Henn. were most susceptible. Pustules of *P. rubigo-vera tritici* (Eriks.) Carleton were least susceptible. Races 17, 38, and 56 of *P. graminis tritici* were equally susceptible to *X. uredovorus*. *Xanthomonas uredovorus* is soil borne. One means of dissemination is by splashing rain. Spread of bacteria to erupted pustules was effected by contaminated urediospores. Bacteria borne on urediospores that initiated the rust infection did not cause bacterial infection.

Bacterial parasites of urediospores of cereal rusts, while uncommon, have been reported in at least 2 instances. Levine et al[3] reported a species of *Bacillus* to be parasitic on urediospores. Borders[4] described a parasitic bacterium on species of *Puccinia* on cereals. He designated the organism *Erwinia urediniolytica*. It attacked only the pedicels of the spores and was not found within the spore wall.

During the summer of 1952, stem rust pustules surrounded by dark water-soaked margins were observed on wheat in a field near Frederick, Maryland. Subsequent examination of the pustules consistently revealed a lysing bacterium, which was found to be parasitic on urediospores of the rust. The characteristics of the causal organism did not agree with those of any previously described species of bacteria. Cultures of the causal bacterium were studied; it was classified; and the mechanism of its action on the uredia of *P. graminis tritici* and on other species of *Puccinia* on cereals was investigated.

Symptoms and etiology.—Typical symptoms of infection by *X. uredovorus* are shown in Fig. 1. The dark brown to black pigmentation around the margin of the uredium often extended into the sorus and was apparent on the reverse side of the leaf at the center of heavily infected pustules. Fewer spores were formed on such pustules than on noninfected pustules. Formation of urediospores ceased on some of the more severely diseased pustules. Large numbers of bacteria were observed immediately surrounding, on, or in the affected urediospores when microscopic mounts of diseased pustules were examined.

Experimental methods and results.—Temperature and moisture play important roles in disease development by *X. uredovorus*. Leaves of plants bearing open pustules were sprayed with a water suspension of bacteria, and the sprayed plants were then incubated in a moist chamber for more than 12 hours. Humidities near 100 per cent were maintained during the moist chamber period. The influence of temperature during and after moist chamber exposure is shown in Table 1. Temperatures near 28°C. during and subsequent to the period in the moist chamber favored rapid development of the bacterium as reflected by symptoms, which appeared 3 days after inoculation. The temperature within the moist chamber was more influential on

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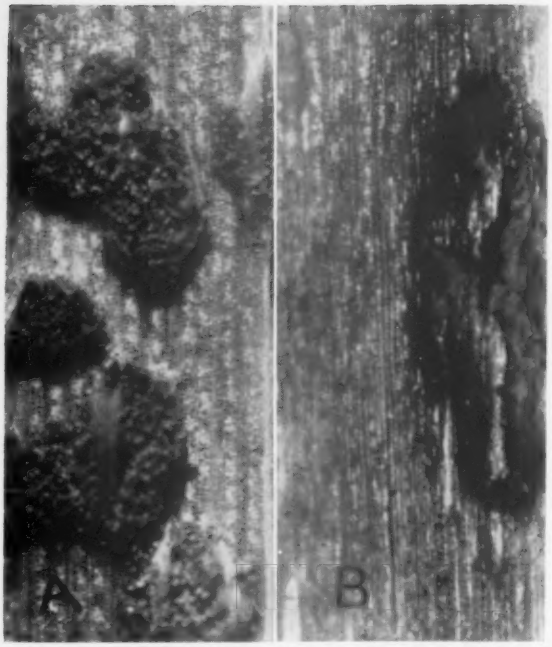

FIG. 1. Symptoms of bacterial attack upon pustules of *P. graminis tritici* on leaves of Baart wheat. A) Normal pustules. B) Affected pustules.

infection than was the greenhouse temperature. Numbers of uredia showing symptoms increased with duration of the period in the moist chamber up to 24 hours at 30°C. The longest moist chamber period employed in these tests was 48 hours at 30°C., but no appreciable increase in infection was observed by extending the moist chamber period beyond 24 hours (Fig. 2).

Wheat plants bearing pustules of different ages were inoculated with bacteria to determine whether age of pustules was a factor in susceptibility to infection. Results are in Table 2. Completely erupted rust pustules appeared to be equally susceptible regardless of age.

TABLE 1.—*The effect of moist chamber and greenhouse temperatures on the parasitism of pustules of P. graminis tritici by X. uredovorus*

Temperature in °C.		Percentage of pustules infected[c]
In moist chamber[a]	In greenhouse[b]	
35[d]	27-36	
30	27-36	93
28	27-36	97
	18-24	97
26	27-36	92
24	27-36	88
21	27-36	91
	18-24	67
16	27-36	75
	27-36	69
13	18-24	51

[a] 24-hour period.
[b] 10-day period.
[c] Averages of 3 successive tests of 30 pots per replication with 10 plants per pot.
[d] Plants wilted.

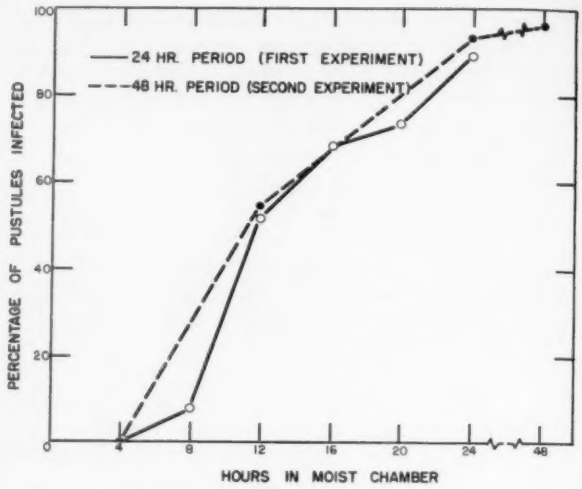

FIG. 2. Relation of period in moist chamber at 30°C to infection of pustules of *P. graminis tritici*.

Koch's postulates have been fulfilled in proving the pathogenicity of *Xanthomonas uredovorus*. The bacterium has been reisolated from infected pustules and subsequent inoculations with the reisolates have incited new infections. Uredia of *P. graminis tritici* on wheat and barley, of *P. graminis avenae* Eriks. and Henn. on oats, and of *P. graminis secalis* Eriks, on rye have become infected in the greenhouse from artificial inoculations. In addition to parasitizing urediospores in pustules on the host, the bacterium has also attacked urediospores germinating on agar surfaces. Urediospores dusted on water agar exhibited lysis within 3 days after inoculation. Nearly all spores were infected by the seventh day (Fig. 3, A). Germ tubes of the spores also appeared to be lysed. Urediospores on water agar sprayed with sterile water remained intact (Fig. 3, B). Urediospores taken from artificially inoculated pustules also revealed lysing (Fig. 3, C) whereas those from healthy pustules remained intact (Fig. 3, D).

Pustules of *P. rubigo-vera tritici* were not so susceptible to attack by *X. uredovorus* as were pustules of *P. graminis tritici*. Of 450 pustules of *P. rubigo-vera tritici* inoculated only 0.4 per cent were attacked in contrast to 85 per cent of 412 pustules of *P. graminis tritici*.

Storage data on *X. uredovorus* indicated a higher

TABLE 2.—*Relation of age of pustules to infection by the bacterium*

Days after inoculation with urediospores	Stage of rust development	Percentage of pustules infected
6	flecking	trace
8	eruption not evident macroscopically	30
13	well-erupted	100
18	well-erupted	97
20	well-erupted	97

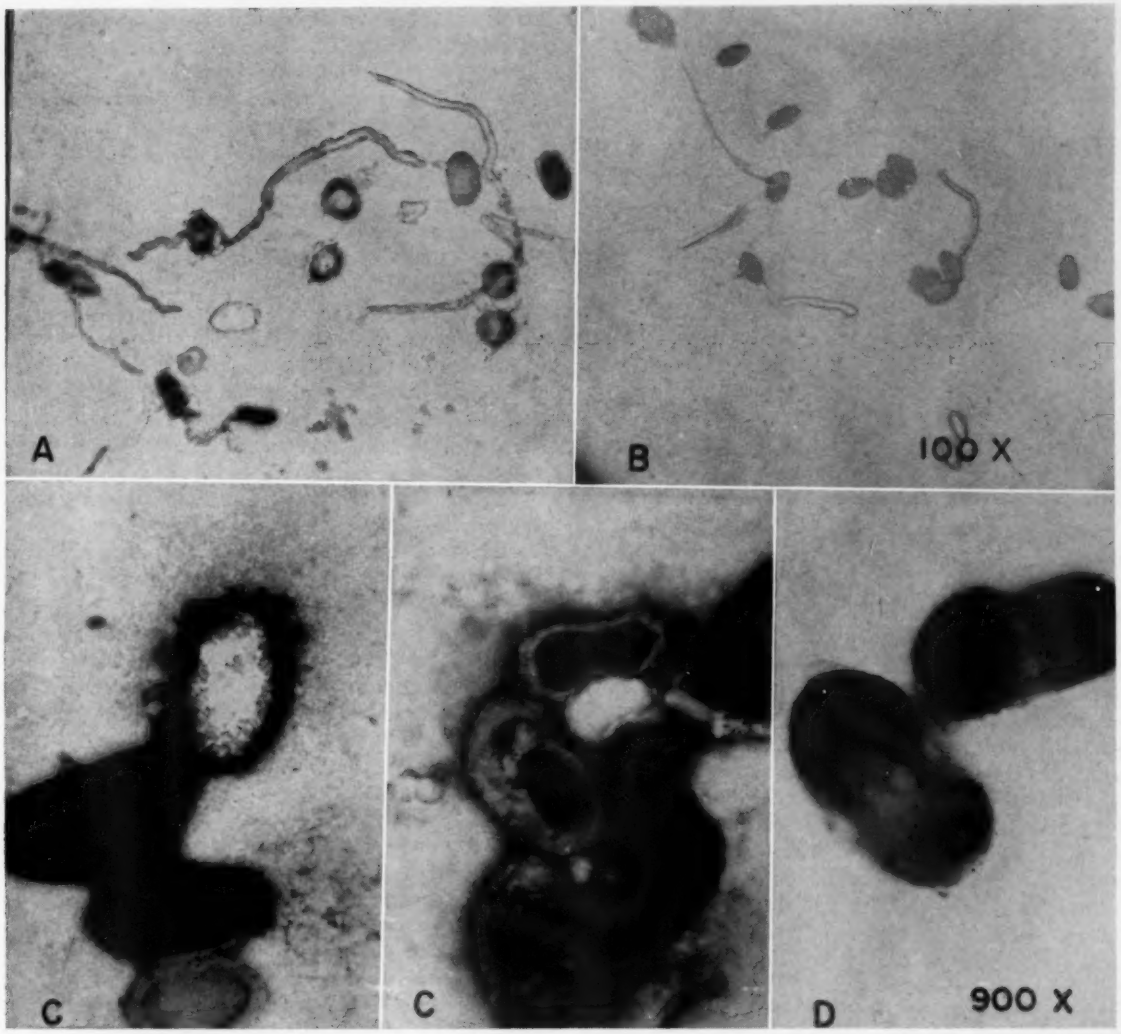

FIG. 3. A) Urediospores on water agar inoculated with *X. uredovorus*. Contents of some of the spores have disappeared and bacteria are visible, within the spores. B) Urediospores on water agar sprayed with sterile distilled water. C) Urediospores in various stages of deterioration removed from a pustule attacked by *X. uredovorus*. D) Urediospores from bacteria-free rust pustule.

degree of stability as to pathogenicity *in vivo* than *in vitro*. Difco nutrient agar cultures stored at laboratory temperatures ranging from 26° to 35°C. during the summer of 1952 lost pathogenicity in about 1 month. Progeny from the non-pathogenic cultures retained the original morphologic characteristics. Three of 10 agar cultures lost pathogenicity after storage for 3 months during the summer of 1953 at laboratory temperatures ranging from 21° to 31°C. However, pathogenic cultures were obtained from infected pustules on dried leaf tissue after storage for 5 months in 1952 at temperatures ranging from 26° to 35°C.

Xanthomonas uredovorus was found to exist as a saprophyte in soil. Sterilized soil was inoculated with bacteria at the time of seeding Baart wheat. Primary leaves of the wheat were inoculated with *P. graminis tritici* and, after pustule development, lysing was noted as a result of transfer of bacteria to the pustules by means of splashing water from the soil surface. Pustules on plants grown in soil that had not been infested with the bacteria did not exhibit symptoms of bacterial attack. Assayed soil samples collected from areas where infected pustules were found in the summer of 1952 yielded lysing bacteria. This is in agreement with the findings of Borders[4].

Experiments were made to determine whether or not various races of wheat stem rust exhibit differences in susceptibility to attack by this bacterium. Races 17, 38, and 56 of *P. graminis tritici* were used for the test. Results showed that the 3 races were equally susceptible to the lysing bacterium. Of more than 300 inoculated pustules examined for each race, more than 90 per cent were infected.

To determine whether or not the bacteria are spread

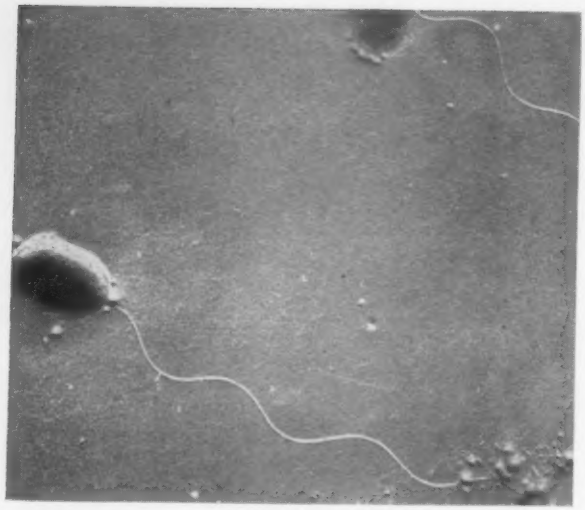

FIG. 4. Electron micrograph of *Xanthomonas uredovorus* sp. nov. showing the single polar flagellum.

by urediospores from infected pustules in the field, such spores were inoculated onto Baart wheat. After pustules had erupted the plants were returned to the moist chamber but symptoms of attack by the bacteria did not develop. Similar negative results were obtained when inoculum was obtained from freshly lysed pustules in the greenhouse. As yet there is no positive proof that bacterial infection occurs from the bacteria which may be present on a urediospore that initiates the rust infection. However, when urediospores contaminated with the bacteria were dusted upon normal pustules and subjected to moisture for 12 hours, infection resulted. This is a method involved in secondary spread of the bacteria in nature. Cereal rusts have been reported to be attacked by bacteria in the field[5] and recently such parasitism has also been observed by the senior writer.

CLASSIFICATION OF THE BACTERIUM.—In the classification of this organism, the usual methods of pure culture study of bacteria were followed. With the exception of its parasitism of *P. graminis tritici*, etc., the bacterium is classified solely by its morphologic and physiologic characteristics. Since it is a motile, gram-negative rod producing a water-insoluble pigment and fermenting most of the usual laboratory carbohydrates with the formation of acid but no gas, it belongs in the family *Pseudomonadaceae* according to Bergey's manual.[6] Because it does not produce a water-soluble pigment, it is a member of the genus *Xanthomonas*. Since the characteristics do not fit any named species in all details, it would appear to be an unnamed species of *Xanthomonas*. It is hereby proposed to designate the organism *Xanthomonas uredovorus*. The complete classification and description of *X. uredovorus* follow:

Xanthomonas uredovorus. sp. nov.

Morphology: Gram negative rods occurring singly, 0.6 × 1.5μ, monotrichous (Fig. 4).

Nutrient agar (Difco) colonies: Slightly raised at center and slightly wrinkled surface, irregular to round, entire edge, orange-yellow surface colonies, ridge at edge.

Nutrient agar (Difco) slant: Orange-yellow, flat, slightly rough with undulated edge.

Nutrient broth (Difco): Very turbid with heavy yellow, slightly flaky sediment, slight pellicle, which settles at slightest agitation.

Potato: Fairly heavy yellow, mucoid growth; no discoloration of potato.

Gelatin colonies: Yellow colonies showing definite liquefaction after 72 hours at 30°C.

Gelatin stab: Slight yellow surface growth with little growth in stab, moderate liquefaction after 7 days at 30°C.

Stone gelatin plate: Positive liquefaction.

Litmus milk: Litmus reduced after 72 hours and milk coagulated after 7 days.

Simmons citrate agar: Sodium citrate utilized as sole source of carbon.

Nitrates reduced to nitrites.

Acetyl methyl carbinol produced.

Methyl red negative.

Lead acetate agar (Difco): Hydrogen sulfide production very slight.

Indole produced.

Yeast extract (1 per cent); starch (0.5 per cent); agar (2 per cent): Starch not hydrolyzed.

Dunham fermentation tubes containing nutrient broth (Difco) base + 0.5 per cent carbohydrate + brom thymol blue indicator. Initial pH 7.0 ± 0.2: Acid but no gas from cellobiose, dulcitol, dextrose, dextrin, fructose, galactose, glycerol, inositol, lactose, maltose, mannitol, mannose, rhamnose, salicin, sorbitol, sucrose, trehalose, and xylose. Acid from arabinose after 72 hours.

Optimum temperature: 30°C.; grows well at 37°C. All the above tests were made at 30°C.

Parastic on pustules of *P. graminis tritici*, *P. graminis avenae*, *P. graminis secalis*, and *P. rubigo-vera tritici*.

DISCUSSION.—*X. uredovorus* differs from previously described bacterial parasites of rusts in being monotrichous, a non-spore former, and of an endoparasitic nature. There are occasions when conditions in nature would permit it to play an important role in biological control of rusts of cereals but because of its high humidity requirements they are extremely rare.

CAMP DETRICK
FREDERICK, MARYLAND

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CONTROL OF SCLEROTIUM ROLFSII SACC. IN IRIS BULBS[1]

Charles J. Gould

SUMMARY

Crown rot of bulbous iris (caused by *Sclerotium rolfsii* Sacc.) is world wide in distribution. Although most serious in warmer climates it has caused considerable damage in western Washington in certain years. Soaking infested bulbs for 3 hours in a warm (110° F.) solution of formalin (0.5 per cent) has given practical control in both experimental and commercial tests. This treatment should be supplemented by sufficient rotation, careful culling, and other sanitation practices.

Sclerotium rolfsii Sacc. is apparently present (10) in all the major bulbous iris-producing areas of the world, causing a bulb disease commonly called crown rot. The fungus is widespread in the United States (12). Usually it is more destructive to iris in warm areas such as eastern North Carolina (5) than in cool ones such as western Washington (4). However, at times serious losses occur in the latter area, particularly in years when rains keep the soil moist towards the end of the growing season (July or August, depending on the area) and soil temperatures are warm. Under such conditions the fungus spreads rapidly through the soil, especially along cracks caused by the enlargement of the iris bulbs. It also grows from bulb to bulb in storage trays under warm, moist conditions.

The symptoms and general control measures of crown rot have been described previously (4). Tests on bulb treatment for the control of the disease in iris are summarized in this paper.

MATERIALS AND METHODS.—Iris bulbs used in the tests came from naturally infested stocks of the varieties Wedgwood and DeWit. They were usually dipped in solutions or suspensions of the materials under test, although in a few cases dusts were applied by rolling the bulbs and fungicide together in a fiber drum. The bulbs were planted in September or October, generally the same day they were treated, in randomized blocks in sandy loam soil near Puyallup, Washington. They were dug the following July or August and yield records obtained. Experiments on bulb treatment were run from 1945 to 1950 and again in 1952–53. Additional details are given with the description of the experiments.

Treatments in cool solutions.—In the first series of tests, bulbs were dipped in moderately warm (80°F.) or cool (55–65°F.) solutions of various materials. The length of time of dipping varied from 5 minutes to 1 hour. The concentration varied with the material, with more than 1 concentration being used in certain cases. Although infested stock was always used in this series of tests, the climatic conditions were unfavorable for development of the fungus, and the disease loss was usually low. Under these conditions the following materials appeared to be ineffective: lime-sulfur, various carbamates, glyoxalidine, lauryl isoquinolinium bromide, phenyl cadmium dilactate, and Omilite. In some tests certain organic and inorganic mercury compounds gave partial control, but in other tests they failed completely. In addition, the mercurials as a group generally reduced yields below the untreated checks. For instance, in the 1946–47 experiments untreated Wedgwood bulbs yielded 52.4 lb. as compared with 36.2 lb. for those soaked on September 27 in mercuric bichloride for 1 hour followed by a 1-minute dip in 2 per cent Ceresan. This treatment has been recommended (8) for blue mold control in bulbous iris. The time of treating is apparently related to injury as stated by McWhorter (8). This is shown by some of our results with Ceresan M in 1948–49 with the Wedgwood variety. Bulbs dipped in Ceresan M (0.17 per cent suspension) on October 8 yielded 17.4 lb. as against 20.9 lb. for comparable ones dipped on August 26.

In contrast to the apparent injurious effect of mercurial compounds was the apparent stimulation by lime-sulfur and various carbamates, although the latter were ineffective in controlling crown rot. Results with the carbamate compounds will be reported later.

Treatments in warm solutions.—In the 1945–46, 1947–48, and 1948–49 experiments, a hot water (110° F.) plus formalin (0.5 per cent = 1 part commercial U.S.P. formaldehyde in 200 parts of water) treatment was included. The treatment was applied late during the storage season in all 3 seasons, and considerable injury resulted. However, sufficient crown rot was present in the last trial on the variety DeWit to indicate that a 3-hour hot water plus formalin treatment had some promise (16.1 per cent loss in the untreated vs. 0.6 per cent in the treated) for controlling the disease. This was substantiated by a commercial grower's observations that crown rot was eliminated in a stock of bulbous iris treated the same way for the control of nematodes. Consequently, the experiments with hot water plus formalin were expanded during the 1949–50 season.

Crown rot-infested bulbs of the variety Wedgwood were treated August 18, 1949, dried, returned to common shed storage until September 21, and then planted in a field in medium sandy loam. They were harvested in July of 1950, cleaned, and held in common storage until examined for disease in August. Results are presented in Table 1. Since the cool bulbs temporarily lowered the temperature, an extra 15 minutes were allowed for the water to return to 110° in all 3 hot water treatments. Four replications of 85 bulbs each were used per treatment.

All treatments significantly increased the number

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TABLE 1.—*Yield of Wedgwood iris bulbs treated in solutions of formalin in 1949, planted, and harvested in 1950*

Treatment	Formalin Per cent	Temperature of Water	Duration of Treatment	Yield of Bulbs: Average number per plot Healthy	Diseased	Total	Average Wt. (lb.) per plot Healthy	Diseased	Total
Untreated	0.0	0	0	7.5	71.2	78.7	0.6	5.1	5.7
Formalin[a]	1.0	55-65°F.	3 hours	46.2	29.5	75.7	4.3	2.0	6.3
Formalin[a]	2.0	55-65°F.	3 hours	56.5	10.2	66.7	4.7	0.5	5.2
Formalin[a]	0.5	110°	1 hour	46.0	30.0	76.0	4.2	2.0	6.2
Formalin[a]	0.5	110°	2 hours	68.0	10.0	78.0	6.1	0.6	6.7
Formalin[a]	0.5	110°	3 hours	72.8	5.5	78.3	6.2	0.4	6.6
L. S. D. 5%				27.1	23.2	N. S.	2.3	1.6	N. S.
1%				37.5	32.1		3.2	2.2	

[a] U. S. P. formaldehyde (37% active)

and weight of healthy bulbs. The 3-hour hot water plus formalin treatment gave the best yield of healthy bulbs, although it was not significantly greater than the 2-hour hot water plus formalin treatment or the cool 2 per cent formalin soak. Since both the cold and warm solutions of formaldehyde were beneficial, the formaldehyde appears to be the effective agent and is presumably activated by the higher temperatures. In connection with the disinfection of potatoes with formaldehyde to control common scab, Melhus and Kent (9) state that better results are obtained when the temperature of the solution is raised.

The recommended treatment in the Pacific Northwest for the control of the bulb or stem nematodes (*Ditylenchus* species) is a hot water (110°) plus formalin (0.5 per cent) treatment of 4 hours for daffodils and 3 hours for bulbous iris. The optimum periods for treating iris have been investigated by Courtney and Gould (3). According to Courtney (2) the formaldehyde in the hot water is essential to complete the nematode kill and prevent the dispersal of certain rot organisms.

Since formaldehyde was apparently more effective in warm than in cool solutions in our tests for the control of crown rot in iris, and growers already had available the necessary equipment for hot water treatment, the 3-hour hot water plus formalin treatment was adopted for trial recommendation to growers for the control of crown rot in bulbous iris.

Reports from growers indicate that this treatment has given excellent control of the disease. Control usually approaches but is not quite 100 per cent effective, because an occasional carry-over may occur. This failure is most often caused by improper treatments, such as exposure of bulbs to treatment for too short time or the use of excessively full sacks of bulbs, in which case the bulbs in the center of the sack apparently are not exposed to the 110° temperature for the full time.

However, the fungus may survive occasionally in large bulbs even after 3 hours of treating, perhaps in the form of extra hard sclerotia. Presumably, soaking bulbs in water prior to the hot water treatment might activate the sclerotia, thereby rendering them more susceptible to treatment. Such carry-over of the fungus was clearly demonstrated in a test in 1952–53 which involved a stock of unusually large bulbs, some of which were affected with crown rot. Part of the stock was left untreated and the remainder was treated in hot water (110°) plus formalin (0.5 per cent) for 1, 2, 3 or 4 hours. The percentage of harvested bulbs infected with *S. rolfsii* was: 48.2 per cent in the untreated; 44.2 per cent in those treated for 1 hour; 24.3 per cent for 2 hours; 21.7 per cent for 3 hours; and 0.7 per cent for 4 hours. The 4-hour treatment would apparently give best control on large bulbs but is too apt to cause injury to permit its general use.

A supplementary fungicide treatment following the hot water treatment appears to be beneficial. For instance, in a test in 1947–48 after Wedgwood bulbs were given the standard 3-hour hot water plus formalin treatment, part of them were dipped 3 hours later in a suspension of thiram (1 lb. Tersan in 8 gal. water). Five replications of 100 bulbs each were used per treatment. The total yield of non-thiram treated bulbs was 14.8 lb. as compared with 18.7 for the thiram-treated.

DISCUSSION.—The 3-hour soak in hot water (110°F.) plus formalin has afforded practical control of crown rot of bulbous iris in both experimental and commercial tests in the Pacific Northwest. Since partial control was obtained in all solutions of formaldehyde, the main benefit is attributed to the formaldehyde, although the possible therapeutic effect of the heat cannot be eliminated on the basis of these experiments.

An interesting aspect of these experiments and observations is the fact that bulbs partially infected with crown rot can recover from the disease if: a) given the proper hot water plus formalin treatment or b) planted in well-drained heavy soil in western Washington. The prevailing temperature in the latter area is probably too low for satisfactory invasion by the fungus. The older fleshy scales of an iris bulb gradually wither during the growing season and are removed when the bulbs are cleaned. Thus the bulb essentially sheds the infected portions under suitable growing conditions without invasion of the new healthy scales occurring.

Although the hot water plus formalin treatment gives satisfactory control of *Sclerotium rolfsii* in iris bulbs, it does not protect them from attack when planted in infested soil. The fungus can survive for many years in the field in the absence of iris by growing on

a wide range of hosts (11) including certain weeds. Therefore, a soil treatment is also needed. Preliminary results indicate that pentachloronitrobenzene is effective, and further tests with it are in progress.

The relation of other hosts that introduce the fungus and maintain it in the soil is important. In addition to iris, the fungus has been found on lilies (1), scilla (6), tulips, and narcissus. Some of these are often used in rotation with iris in the Pacific Northwest. Narcissus has frequently been a carrier of the fungus in this area and also, according to Haasis,[2] in the eastern United States. The fungus seldom injures narcissus bulbs, usually confining its invasion to the outer dead scales, but in such scales it is carried from infested to non-infested fields. Hence, to assist in obtaining complete control, the fungus should be eliminated from these rotation crops by: a) treating narcissus bulbs in hot water plus formaldehyde for 4 hours, and b) extremely careful culling of the other bulbs, since a satisfactory hot water plus formalin treatment for them has not yet been determined.

The steps necessary for control of this disease in bulbous iris are: a) discard all obviously diseased bulbs; b) treat the others for 3 hours in hot water (110°) plus formalin (0.5 per cent); c) plant on well-drained noninfested soil (or perhaps on infested soil treated with pentachloronitrobenzene); d) take proper precautions to prevent spread of the fungus from infested to noninfested fields; and e) use noninfested plants in the rotation cycle.

Sclerotium rolfsii has been adopted as the name for the causal agent for this paper in view of the recent work by Lyle (7) indicating that the large sclerotial form consistently encountered here and previously called *S. delphinii* Welch is merely a biotype.

These results suggest the possibility of curing other plants infected with *Sclerotium rolfsii* by a hot water plus formalin treatment.

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STATE COLLEGE OF WASHINGTON
WESTERN WASHINGTON EXPERIMENT STATION
PUYALLUP, WASHINGTON

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THE REACTION OF SEVERAL CARNATION VARIETIES TO BACTERIAL WILT[1]

W. D. Thomas, Jr.

SUMMARY

The reactions of 5 carnation varieties to infection by *Pseudomonas caryophylli* Burkholder have been investigated. The Durango variety was apparently immune from bacterial wilt, and had no tendency to carry the causal organism. White Sim and Skyline carnations were moderately resistant carriers of the bacterial wilt pathogen. Arapahoe and Miller's Yellow varieties were susceptible and could not be considered as carriers of the bacteria. It was evident that carnation varieties resistant to bacterial wilt might serve as symptomless carriers of the pathogen.

Since its description in 1941 (4), bacterial wilt has become a factor limiting carnation production throughout the United States. Its rapid and wide dissemination by carnation cuttings was indicated by Creager (2). In his discussion of the disease, Jones (4) reported that cuttings taken from plants prior to the appearance of symptoms were responsible for transmitting the pathogen. No mention was made, however, that certain carnation varieties may serve as carriers of the wilt bacteria.

Following Lutman's observations (6, 7) that entire potato tubers may be infected with *Actinomyces* hyphae, several other investigators have reported micro-

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TABLE 1.—*Reaction of 5 carnation varieties to inoculation with Pseudomonas caryophylli*

Variety	No. plants inoculated	No. wilted	No. vascular discoloration	No. + isolations	Resistance[1]
Durango	100	0	0	0	I
White Sim	100	6	19	89	MRC
Skyline	100	6	28	100	MRC
Arapahoe	100	50	100	100	S
Miller's Yellow	100	70	100	100	S

[1] I, immune; MRC, moderately resistant carrier; S, susceptible.

organisms in presumably sterile plant tissues. Armstrong and Armstrong (1) found that nonsusceptible hosts may serve as carriers of wilt Fusaria. Similar observations were made with carnation Fusaria by Thomas and Baker (11). Hollis (3) found 14 types of bacteria, mostly nonpathogenic, in potato tissue. Similar results were obtained by Sanford (9) and Thomas and Graham (12).

Thomas (10) found that temperature may be a deciding factor in determining the wilt expression by carnation carriers of *Fusarium oxysporum* f. *dianthi* (Prill. et Del.) Sny. & Han., with some correlation between varieties. Pierson (8) reported that the tissues of all varieties of cucumbers investigated by him were penetrated by *Cladosporium cucumerinum* Ell. & Arth. after inoculation; the fungus was initially intercellular, but it shifted to an intracellular nature principally in susceptible varieties. Intracellular infection was rare in resistant varieties. In determining the reaction of certain potato varieties to ring-rot infection, Lane (5) observed that the resistant Teton potato variety served as a very efficient carrier of *Corynebacterium sepedonicum* (Spiek. & Kott.) Skap. & Burkh.

Two serious outbreaks of bacterial wilt in Denver greenhouses in 1951 called attention to the previous work which had been done by other workers on plant carriers of pathogens. In one greenhouse a 1-year old bench of William Sim carnations served as a mother block for propagation. In another greenhouse a 2-year old bench of the Skyline variety served as a mother block. The mother plants appeared healthy and vigorous, and had a good production record. Despite the employment of all known practices of good sanitation, 79 per cent of the William Sim and all the Skyline cuttings succumbed to bacterial wilt. A recheck of the mother plants 1 month later, in July, showed no indication of wilt symptoms, but randomized isolations made from the plants always yielded the pathogen, *Pseudomonas caryophylli* Burkholder.

In view of these outbreaks and the potential hazard of bacterial wilt epiphytotics arising from an intensive cutting-improvement and production program inaugurated by the Colorado Flower Growers Association, it seemed necessary to determine whether some carnation varieties may serve as symptomless carriers of the pathogen, and to investigate the reactions of certain commonly-grown carnation varieties to the causal organism.

MATERIALS AND METHODS.—Cuttings of White Sim, Durango, Arapahoe, Skyline, and Miller's Yellow carnations were obtained from mother plants which previously had been found free of any pathogens. This was determined by standard indexing procedures incorporating the isolation of tissues obtained from the root crown.

The inoculum used in this investigation was increased from a single-colony culture of *P. caryophylli* obtained by the dilution-plate method. It was maintained in beef-peptone broth at a temperature of 30°C. Inoculum was from 5-day-old cultures.

The soil used in this investigation was tested at pH 6.6, and was rich in organic matter. It had been pasteurized at approximately 170°F. 48 hours prior to planting.

Two hundred cuttings of each of the 5 varieties were rooted in pasteurized sand. At the time of transplanting to sterilized 4-in. pots, the roots of 100 of the cuttings of each variety were dipped into a beef-peptone broth suspension of the bacteria. The remaining cuttings served as non-inoculated checks. After 5 weeks all plants were pinched to favor the development of more breaks which might be likely to indicate wilt symptoms. The plants were subjected to greenhouse temperatures averaging 70°F. during the day, and 60°F. at night from the time of transplanting throughout the investigation.

At the end of 6 months, observations were made of wilt symptoms and vascular discoloration in each plant. Isolations on potato-dextrose agar were made from the root crown of each plant to determine the presence of the pathogen.

EXPERIMENTAL RESULTS.—The results shown in Table 1 indicated that no symptoms of the disease were evident in any of the Durango plants, and that the organism could not be isolated from any of the plants. From all indications it seemed apparent that the Durango variety was immune from the culture of *P. caryophylli* used in this investigation.

Six plants each of the White Sim and Skyline carnations wilted. Of the former variety, 19 plants had vascular discoloration at the root crown, and 28 plants of Skyline showed evidence of vascular infection. All isolations from the Skyline plants yielded the pathogen, whereas 89 of the White Sim plants contained the organism. The indications were that both varieties were moderately resistant to the pathogen, and that both varieties might serve as efficient carriers of the pathogen.

Half of the Arapahoe plants wilted by the end of the investigation, but all of them had discolored vascular

systems. All isolations from this variety were positive. Because of the evidence of vascular infection in all the test plants, the variety could not be considered very resistant to the disease, but because of the failure of half of the plants to wilt during the period of the test, there was some indication of slight resistance. Close scrutiny of the plants at the time of cutting, however, indicated that the Arapahoe carnation variety could not be considered a true symptomless carrier of the bacterial wilt pathogen.

Of the Miller's Yellow plants in this test, 70 completely succumbed to the disease, the remaining 30 not appearing so vigorous as did the check plants. All of them had vascular discoloration and yielded the organism upon isolation. This variety was rated as completely susceptible to bacterial wilt, with no tendency to serve as a symptomless carrier of the pathogen.

None of the check plants of any variety showed any evidence of bacterial wilt, and all isolations from the plants were negative.

COLORADO AGRICULTURAL AND MECHANICAL COLLEGE
FORT COLLINS, COLORADO

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ROD-SHAPED PARTICLES ASSOCIATED WITH LETTUCE MOSAIC[1]

Houston B. Couch[2] and A. Herbert Gold

SUMMARY

Flexuous, elongated particles, averaging 22mμ × 746mμ, were found in association with lettuce mosaic virus in 2 commercial varieties of lettuce, in the floral and vegetative tissue of *Zinnia elegans* and *Tagetes erecta*, in the vegetative tissue of *Cichorium endivia* and *Callistephus chinensis*, and in local lesions on *Gomphrena globosa*. In each case, samples taken from mosaic-free plants failed to reveal such particles. Concentration of these particles in the droplet patterns was less than 10^{10} per gm. fresh weight. Utilization of methods capable of increasing infectivity of the virus inoculum preparations failed to give any measurable increase in number of particles recovered from the host tissue.

A study of rod-shaped particles associated with the common lettuce mosaic virus was conducted with the electron microscope. These particles were observed in crude preparations of lettuce and other host plant tissue prepared according to the technique described by Backus and Williams.[3] Weighed leaf samples were finely ground in a mortar, in the presence of a little distilled water to facilitate grinding, after which enough water was added to make a dilution of 1:90 on a weight basis. One tenth ml. of Dow polystyrene latex (lot 15 H-8, Physical Research Laboratory), containing 1.2×10^{11} spheres per ml., was added to 0.9 ml. of the homogenate, and this mixture was sprayed onto collodion-coated screens. The final step, prior to study, consisted of shadow-casting the material with palladium at an angle of 15°.

The initial sources of the lettuce mosaic virus were seed-borne, mosaic-diseased lettuce plants, of the varieties Bibb and Great Lakes. Preparations from over 12 each of healthy and diseased lettuce plants were compared in the electron microscope. In the preparations from infected plants, flexuous, rod-shaped particles, as shown in Figure 1, were observed. Similar structures were not found in healthy tissue. Forty-

[1] Accepted for publication August 16, 1954.

[2] Former Research Assistant, Department of Plant Pathology, University of California at Davis; now Assistant Professor of Plant Pathology, Pennsylvania State University, State College, Pennsylvania.

[3] Backus, R. C. and R. C. Williams. 1950. The use of spraying methods and of volatile suspending media in the preparation of specimens for electron microscopy. Jour. Appl. Physics 21: 11-15.

FIG. 1. Electron micrograph of flexous, rod-shaped particles from a lettuce plant infected with the common lettuce mosaic virus. Shadow cast with palladium; polystyrene sphere, 514 mμ in diameter, used for calibration.

five of these rod-shaped particles were measured, using the Dow polystyrene latex spheres as a standard. These particles averaged 22 mμ in diameter and 756 mμ in length. The standard deviation of the length measurements was 13.2 mμ with a standard error of 2 mμ.

Preparations were also made from the following hosts: zinnia, *Zinnia elegans*, Jacq.; marigold, *Tagetes erecta*, L.; endive, *Cichorium endivia*, L.; aster, *Callistephus chinensis*, Nees; and flowering amaranth, *Gomphrena globosa*, L. Plants of these species were inoculated by mechanical means with inoculum from seed-borne mosaic lettuce plants. Preparations for study in the electron microscope were made from both mosaic-diseased and healthy plants. Particles of the same shape and size as those found in mosaic-diseased lettuce were also observed in the preparations from infected plants of all species examined. These structures were not present in the healthy controls. In the cases of zinnia and marigold, particles were found both in leaf and petal tissue of infected plants. New local lesions of inoculated *G. globosa* plants were found to contain the rod-shaped particles, but inoculated leaf tissue immediately outside the local lesions were free of similar particles. Since the infection in *G. globosa* may later become systemic, particles might have been observed outside the local lesions on old infections but these were not examined.

Using the method of Backus and Williams,[3] an attempt was made to determine the concentration of the particles in the respective hosts. The apparent concentration was so low (less than 10^{10} per gm., fresh weight) that an accurate determination could not be made. However, the concentration in zinnia ray flowers and in local lesions of *G. globosa* appeared to be somewhat higher than in the tissue of other hosts. This observation was not confirmed by actual count, since the concentration of particles was still too low for accurate measurement.

Ainsworth and Ogilvie[4] reported an increase in percentage infection with lettuce mosaic virus when 0.5 per cent sodium sulfite was used in the inoculum; Hirsch[5] found that for the reducing agent to be most effective, it should be added before the material is ground. In an attempt to determine whether sodium sulfite increased the concentration of particles observed in the electron microscope, the material was added to some samples before homogenization. The final concentration of the added sulfite was 0.1 per cent. No obvious increase in particle concentration was observed; however, concentration with all treatments was too low for accurate determination.

Wilkinson and Hirsch[6] reported that *Gomphrena globosa* and *Chenopodium urbicum* are local lesion hosts of the common lettuce mosaic virus and that these plants are suitable for quantitative virus assay. A preliminary study of the influence of dark exposure of infected lettuce plants on virus activity, using *G. globosa* as the local lesion assay host, has shown a significant increase in infectivity at the 5 per cent level when the source of inoculum was held in the dark for 8 hours. However, when seed-borne mosaic plants of variety Bibb were given an 8-hour dark treatment prior to sampling for electron microscope preparations, no obvious increase in the number of particles was evident. An attempt was made to concentrate the particles in the expressed plant sap by centrifugation. This approach was unsuccessful, however, for it was found that even relatively brief, low speed centrifugations resulted in the loss of practically all the particles.

Since the virus is seed-borne, 50 Bibb lettuce seeds from a lot known to average 14 per cent seed-borne mosaic were examined individually in an attempt to correlate presence of the rod-shaped particles in the seed with transmission of the virus. Particles were not observed in any of the seed preparations. One hundred seeds from the same lot were planted, and preparations for study in the electron microscope made from each of 8 seedlings showing mosaic symptoms and 50 seedlings that appeared to be free of the disease. All 8 seedlings showing symptoms contained the rod-shaped particles; whereas, the healthy seedlings were free of them.

DISCUSSION.—Presence of the flexuous, rod-shaped particles in preparations from mosaic-diseased lettuce plants, as well as 5 other species infected with the virus, coupled with the failure to observe similar particles in juice from healthy plants, is definite evidence of their association with the disease. While presently

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[5] Hirsch, Ursula. 1953. Quantitative studies on the lettuce mosaic virus. Master's Thesis. Dept. of Plant Pathology, Cornell University, Ithaca, N. Y.

[6] Wilkinson, R. E., and Ursula Hirsch. 1952. Local lesion hosts for the lettuce mosaic virus. (Abs.) Phytopathology 42: 478.

it cannot be said that these particles are actually virus, they are relatively uniform in length, and their general morphology is similar to that of particles recognized as causal agents of certain other virus diseases. Therefore, we believe that these structures are probably lettuce mosaic virus.

Two factors contributed to the inability to determine concentration of the particles. First, lettuce mosaic virus is relatively unstable. This is evidenced by the difficulty experienced in obtaining a consistently high degree of mechanical transmission of the virus. Even with the use of sodium sulfite, the percentage of successful infections from one trial to the next may vary considerably. This suggests that if the particles do actually constitute the causative agent of the disease, many may disappear even in the short time required for electron microscope preparations. The second factor is an apparent tendency for the particles to occur in clumps of bundles similar to that shown in Figure 1. Such an arrangement might reduce the chances for observing the particles, and could possibly increase any tendency for the particles to sediment out of preparation.

Considering the low concentration observed in leaves of diseased plants, the failure to recover these rod-shaped particles in seed preparations may have been due to an even smaller number of particles in the seed. However, the presence of these characteristic particles in mosaic-diseased seedlings, and their absence in healthy plants from the same seed lot, is further evidence of their association with the disease.

Department of Plant Pathology
University of California
Davis, California
and
Berkeley, California

A PHYTOPATHOLOGICAL NOTE

A flower blight of camellias in North Carolina incited by Sclerotinia sclerotiorum.[1] N. N. Winstead and Frank A. Haasis.[2] Near Wilmington, North Carolina, early in December, 1953, numerous apothecia of *Sclerotinia sclerotiorum* (Lib.) DBy. were observed beneath plants of camellia. This observation suggested a causal relationship between *S. sclerotiorum* and flower blight of camellia. The fungus was subsequently isolated from naturally blighted flowers of the following varieties: Candidissima, Duchess of Southerland, Flame, Herme, Orton Pink, and Pink Perfection. Previously, *S. sclerotiorum* has been reported on *Camellia japonia* L. from Chile[3] and on flowers of *Camellia* spp. in southeastern United States.[4] The purpose of this investigation was to establish the relationship of *S. sclerotiorum* to flower blight of camellia and to compare the disease with that incited by *S. camelliae* Hara.[5, 6]

In the laboratory, inoculations were made with ascospores and mycelia of both fungi, whereas in the field, inoculations to flowers *in situ* were with *S. sclerotiorum* alone, thus to avoid the possibility of spreading *S. camelliae* to a noninfested locality. Inoculations with ascospores were made in the laboratory by 1) placing disease-free blossoms beneath mature apothecia attached to the tops of glass humidity chambers, or 2) spraying blossoms with a water suspension of ascospores. Apothecia of *S. sclerotiorum* employed in the inoculations were collected from beneath camellia bushes and from a field of collard plants, those of *S. camelliae* only from beneath camellia plants. Mycelia used in the inoculations were derived directly from ascospores or by isolation from infected flower tissue. Inoculations to flowers *in situ*, under field conditions, were made either by spraying blossoms with a water suspension of ascospores or by placing bits of mycelium on the petals. The non-inoculated and inoculated blossoms were enclosed in plastic bags to provide isolation and to maintain high humidity.

Blighted flowers consistently followed inoculation with *S. sclerotiorum* under both laboratory and field conditions, irrespective of the method of inoculation. In the laboratory, inoculations with ascospores of *S. camelliae* were always successful but inoculations with mycelia were only occasionally successful. Each fungus was invariably reisolated from the inoculated blossoms and never recovered from the accompanying controls, although *Pestalotia* spp. were encountered occassionally in the latter.

Although early symptoms produced by the 2 organisms were indistinguishable, advanced symptoms were usually distinct. Young lesions produced on the petals by both pathogens appeared about 48 hours after inoculation as small, brownish specks with irregular margins. Under moist conditions, these specks rapidly increased in size and united to form large spots which soon involved the entire flower. The lesions on blossoms

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[2] Respectively Assistant Plant Pathologist, Vegetable Research Laboratory, Castle Hayne, North Carolina, and Professor of Plant Pathology, North Carolina State College, Raleigh, North Carolina.

[3] Anon. 1941. Principalis infermedades parasitaris que objecto de consulta en el segundo Semestre (Julio-Diciember) de 1941. Bol Sanid veg. Santiago. i, 2: 52-55. (Abs. in Rev. Appl. Mycol. 22: 241. 1943.)

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inoculated with *S. sclerotiorum* usually enlarged more rapidly than those incited by *S. camelliae*. In advanced stages of the disease, mycelia and sclerotia could be regularly and conspicuously observed on the surface of necrotic petals when blossoms inoculated with *S. sclerotiorum* were maintained under very humid conditions. Neither mycelia nor sclerotia occurred on the surface of flowers infected with *S. camelliae* at any humidity level. Black, wet masses of microconidia, however, were frequently observed under conditions of high humidity.

The petals of blossoms infected with *S. camelliae* appeared netted; the veins were dark brown and thus rendered prominent in contrast to the lighter brown of the interveinal areas. On flower petals infected with *S. sclerotiorum* such symptoms, on the other hand, were usually less marked or not evident. In 3 cases, however, *S. sclerotiorum* was obtained in isolations from naturally infected flowers that showed symptoms indistinguishable from those produced by *S. camelliae*.

The 2 organisms were easily differentiated in culture. Young cultures of *S. sclerotiorum* were always white with abundant aerial mycelium, whereas those of *S. camelliae* were greyish white with little aerial mycelium. Cultures of *S. sclerotiorum* formed 15–28 sclerotia per petri plate of potato-dextrose-agar medium within 3–4 days at room temperature. The sclerotia normally were formed on the surface of the medium. Cultures of *S. camelliae*, under similar conditions, formed sclerotia only after 10–14 days of growth. Furthermore, the sclerotia per plate were sparse (about 1–7) and for the most part either embedded within the medium or very slightly raised. Microconidia were abundant in cultures of *S. camelliae*, the spores accumulating in dark, beady viscous droplets. Microconidia in *S. sclerotiorum* were few and relatively inconspicuous. Cultures of *S. sclerotiorum* remained white on aging, whereas those of *S. camelliae* turned dark within 2 weeks.

The 2 organisms can usually be differentiated in the field on the basis of the appearance of sclerotia and apothecia. The sclerotia of *S. camelliae* were frequently petalloid and very large, whereas those of *S. sclerotiorum* observed under camellia bushes or associated with diseased flowers were always small and somewhat rounded and elongate. The apothecia of *S. camelliae* appeared to be somewhat darker brown than those of *S. sclerotiorum* and frequently larger. The number of apothecia formed from a single sclerotium was usually low with *S. sclerotiorum*, high with *S. camelliae*, though in both cases quite variable. As many as 45 apothecia have been observed arising from a single large sclerotium of *S. camelliae*, which far exceeded the maximum number produced by *S. sclerotiorum*.

The results show that *S. sclerotiorum* is capable of inciting a flower blight disease of camellia similar to that incited by *S. camelliae*. The 2 diseases usually can be differentiated by symptomatology. Equivocal cases can be resolved by use of isolation techniques, for the 2 organisms are easily differentiated on the basis of cultural characteristics.

The importance of *S. sclerotiorum* as a cause of flower blight of camellia is not known, particularly outside the area dealt with in this instance. Inasmuch as this organism is known to be widely distributed, and, furthermore, is known to be pathogenic to a large number of host plants, one should suspect it of being a rather general pathogen of camellia.—Vegetable Research Laboratory, Castle Hayne, North Carolina, and Plant Pathology Laboratories, Raleigh, North Carolina.

REPORT OF THE 46TH ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The American Phytopathological Society held its 46th Annual Meeting on August 25–27, 1954, at the YMCA Conference Camp, Estes Park, Colorado, in conjunction with the Pacific Division of APS and with the Potato Association of America. The Council met on August 24, 25, and 27 and Society Business meetings were held August 25 and 27. About 1100 people were in attendance, including about 525 members. A total of 208 formal papers of original research were presented in addition to several invitational Symposium papers. Two general Symposia were held: 1) Concepts and Problems in Virology, and 2) Concepts and Problems of Nematode Diseases In Plants. One joint session was held with the Potato Association of America. Informal Conferences were held on 1) Seed Treatment, 2) Oak Wilt, 3) National Tomato Disease Screening Program, 4) Stone Fruit Viruses.

The annual banquet was held as a Chuck Wagon dinner in a colorful western atmosphere. Over 1000 persons attended. Entertainment, which was particularly colorful and appropriate, was presented by plant pathologists of the University of Wyoming.

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C. W. BOOTHROYD, Business Manager; L. C. KNORR, Editor; W. C. PRICE, A. FRANK ROSS (ex-officio), J. C. WALKER.

Placement
E. E. HONEY, Chr.; L. S. HITCHNER, G. C. KENT, G. W. KEITT, J. B. KENDRICK, SR., C. L. LEFEBVRE, H. E. WHEELER.

Public Relations
P. R. MILLER, Chr.; G. H. HEPTING, T. H. KING, G. K. PARRIS, THOMAS SPROSTON, R. A. YOUNG, G. A. ZENTMYER.

Plant Disease Prevention
E. B. LAMBERT, Chr.; S. B. FRACKER, E. C. STAKMAN, E. E. WILSON.

Regulatory Work and Foreign Plant Diseases
J. G. DICKSON, Chr.; E. C. BLODGETT, E. G. REX, G. L. STOUT, W. H. WHEELER, R. P. WHITE.

Seed and Plant Material Certification
JOHN HARDISON, Chr.; C. H. ARNDT, K. F. BAKER, WAYNE BEVER, REINER BONDE, L. C. COCHRAN, J. T. MIDDLETON, W. H. WHEELER.

Special Committees:

Fungicide Colloquium
L. G. UTTER, Chr.; S. I. COHEN, J. W. HEUBERGER, L. S. HITCHNER, R. H. WELLMAN, J. D. WILSON.

Industrial Relations
R. H. WELLMAN, Chr.; C. J. KRISTER, L. G. UTTER, F. L. WINTER, S. G. YOUNKIN.

Membership
GEORGE FISCHER, Chr.; ROBERT CAMPBELL, R. G. GROGAN, D. J. HAGEDORN, W. J. HOOKER, ARTHUR KELMAN, SAUL RICH (ex-officio), D. A. ROBERTS, J. R. SHAY, P. M. SIMMONDS.

Advisory Committee on Nematology
A. L. TAYLOR, Chr.; M. W. ALLEN, J. R. CHRISTIE, M. B. LINFORD.

Nomenclature and Classification of Plant Viruses
A. FRANK ROSS, Chr.; C. W. BENNETT, L. M. BLACK, L. C. COCHRAN, R. W. FULTON, K. W. KREITLOW, H. H. MCKINNEY, F. P. MCWHORTER, W. C. PRICE, T. E. RAWLINS.

Subcommittee on Virus Type Culture Collection
H. H. MCKINNEY, Chr.; R. W. FULTON, T. J. GRANT.

Publication of Special Material
G. L. MCNEW, Chr.; W. H. BRAGONIER, C. C. ALLISON.

Teaching of Plant Pathology
W. H. BRAGONIER, Chr.; D. C. ARNY, C. W. ELLETT, R. J. GREEN, JR., M. F. KERNKAMP, L. D. LEACH, J. H. OWEN.

Utilization of Plant Pathologists and Facilities in National Emergencies
H. H. THORNBERRY, Chr.; A. L. ANDERSON, H. T. COOK, M. F. KERNKAMP, C. H. KINGSOLVER, J. G. LEACH, C. L. LEFEBVRE, H. REX THOMAS.

Temporary Committees:

Advisory Committee on Collecting and Disseminating Data on New Fungicide Tests
L. G. UTTER, Chr.; J. W. HEUBERGER, P. R. MILLER, R. H. WELLMAN, D. A. ROBERTS.

Report of the Secretary. On August 15, 1954, the total membership was 1,650, consisting of 125 Life Members, 15 Emeritus Members, 4 Life and Emeritus Members, 1,489 Regular Members, and 17 Sustaining Associates. Included in the Regular Members are 125 Member Applicants, 54 of whom are students. Since the last meeting reports have been received of the deaths of Ralph E. Smith, F. D. Heald, and E. M. Freeman, charter members, and of G. F. Miles, C. M. Tucker, and E. E. Hubert, J. H. Muncie, and Herbert G. Zuckerman.

GLENN S. POUND, *Secretary.*

Report of the Treasurer.

Receipts:		
Balance on hand, July 1, 1953	$7,255.64	
Membership dues	2,546.00	
Rockefeller Foundation Grant for Symposium on Cooperative Agricultural Research in the Western Hemisphere held at Annual Meeting (Madison)	5,105.00	
Contributions from Industry for publication of Fungicide Data Summaries	2,300.00	
Banquet Fund	363.13	
Total Receipts		$17,569.77
Expenditures:		
Office of Secretary	$ 514.99	
Office of Treasurer	908.98	
Expenses, Placement Committee	36.79	
Expenses, Annual Meeting (Madison)	5,438.19	
Expenses, Publication of Fungicide Data Summaries	501.28	
Expenses, Public Relations Committee	155.00	
Expenses, Fungicide Nomenclature Committee	33.96	
Dues, A.I.B.S.	1,000.00	
Donation, Publication of Catalog of Plant Viruses	200.00	
Registration Fee, Representative to Agric. Res. Inst.	8.75	
Total Expenditures		$ 8,797.94
Balance on hand, June 30, 1954		$ 8,771.83

SAUL RICH, *Treasurer.*

Report of the Business Manager for *Phytopathology*.

Receipts:		
Balance on hand, July 1, 1953	$14,202.64	
Subscriptions and member-subscriptions	22,929.49	
Advertisements	4,583.48	
Reprints and Excess Illustrations	6,018.24	
Sales of Back Issues	1,234.85	
Sustaining Associate Memberships	1,500.00	
Donation, Rockefeller Institute	600.00	
Donation, A. B. Burrell (Life Member)	10.00	
Interest on Investments	693.72	
Total Receipts		$51,772.42
Expenditures:		
Manufacture and Distribution of PHYTOPATHOLOGY	$17,384.23	
Cost of Reprints	1,815.87	
Office of the Business Manager	786.65	
Office of the Editor-in-Chief	2,899.91	
News Letter	6.20	
Office of the Advertising Manager	541.88	
Cost of New Investments	10,435.34	
Collector of Internal Revenue	854.68	
Refunds	157.52	
Miscellaneous	7.60	
Total Expenditures		$34,889.88
Balance on hand, June 30, 1954		$16,882.54

SAUL RICH, *Business Manager.*

Invested funds of The American Phytopathological Society and *Phytopathology*.

Amounts as of June 30, 1954. Dividends and Interest for Fiscal Year July 1, 1953-June 30, 1954.

Sinking Fund:

U. S. Savings Bond, Series G (2½%) (Interest—$25.00)	$ 1,000.00
U. S. Savings Bonds, Series F (at maturity)	1,150.00
Northwestern Fed. Sav. & Loan Ass'n. (Certificate) (Div.—$60.00)	2,000.00
Family Finance Corp., common, 7 sh. at par. (Div.—$9.80)	7.00
Westinghouse Electric Co., 22 sh. at cost (Div.—$44.00)	849.08
Standard Oil of Ohio, common, 50 sh. at cost (Div.—$120.00)	2,018.65
American Cyanamid Co., common, 40 sh. at cost (Div.—$88.65)	2,160.47
Johns-Manville, common, 25 sh. at cost (Div.—$106.25)	1,664.27
New Haven Savings Bank, on deposit (Int.—$2.37)	193.23
Total	$11,042.70

Lyman Memorial Fund:

Westinghouse Electric Co., common, 35 sh. at cost (Div.—$70.00)	$ 1,350.81
Amer. Tel. & Tel., common, 16 sh. at cost (Div.—$122.77)	2,490.47
Total	$ 3,841.28

***Phytopathology* Contingency Fund:**

Johns-Manville, common, 7 sh. at cost (Div.—$29.75)	$ 466.00
Jefferson Fed. Sav. & Loan Ass'n. (Int.—$21.15)	721.57
District Bldg. & Loan Ass. (Int.—$63.37)	2,160.01
Perpetual Bldg. & Loan Assn. (Int.—$42.35)	1,443.37
Arlington & Fairfax Sav. & Loan Ass'n. (Int.—$336.54)	9,868.93
Prudential Bldg. Ass'n. (Int.—$48.25)	1,605.39
Arundel Fed. Sav. & Loan Ass'n. (Int.—$317.74)	9,317.74
Southern Fed. Sav. & Loan Ass'n. (Int.—$284.93)	8,354.93
Decatur Fed. Sav. & Loan Ass'n. (Int.—$122.50)	6,105.00
Fidelity Fed. Sav. & Loan Ass'n. (Int.—$81.86)	4,081.86
Total	$44,124.80

SAUL RICH, *Treasurer and Business Manager.*

Report of the Auditing Committee. On July 23, 1954, an examination was made of the books of The American Phytopathological Society and of PHYTOPATHOLOGY covering the period July 1, 1953–June 30, 1954. All receipts, expenditures, and funds, were found to be accurately recorded and in order.

JAMES G. HORSFALL, Chr.
J. S. TIDD.

Report of the Advertising Manager. From October, 1953, through September, 1954, there were 96.5 advertising pages with 115 paying advertisements. The gross income was $4645.50, with a balance of $4221.53 after agency commissions and cash discounts were deducted. Expenses of the advertising manager were postage and telegraph charges of $51.37 and $72 for stenographic assistance. The commission of the advertising manager amounted to $372.15 making a total of $495.52 in expenses.

Total income to the Society is $3,726.01.

LEONARD J. ALEXANDER, *Advertising Manager*

Report of the Business Manager of Phytopathological Classics. The number of copies of Phytopathological Classics sold during the fiscal year was 130, having a total value of $179.75. Number of classics on hand June 30, 1954; No. 1, none; No. 2, none; No. 3, 50; No. 4, 132; No. 5, 349; No. 6, 404; No. 7, 348; No. 8, 533; No. 9, 763.

Receipts:

Cash balance on October 1, 1953		$ 416.58	
Received during fiscal year 1953-54:			
From sale of Classics	$ 179.75		
On accounts due	211.75		
Overpayment for Classics	9.38	400.88	$ 817.46

Disbursements:

Postage	$ 10.00		
Refund of overpayment for Classics	.50	$ 10.50	$ 817.46
Cash balance on June 30, 1954		806.96	

Due on accounts June 30, 1954:

Present fiscal year	$ 14.25
Previous fiscal year	35.00
Total due on accounts	$ 49.25

CARL W. BOOTHROYD, *Business Manager.*

Report of the Editor of Phytopathological Classics. The last number in the *Phytopathological Classics* series, issued in 1952, was the Tehon translation of Targioni Tozzetti's ALIMURGIA. Since then several manuscripts have been proposed for printing, but in keeping with PC's attempts at self-sufficiency, their publication must await funds from the sale of previous issues.

Suggestions for future inclusions in the PC series continue to be welcomed.

L. C. KNORR, *Editor.*

Report of the Editor-in-Chief. The earliest editor's report to be found in PHYTOPATHOLOGY, that in Volume 8, calls attention to the need for care in preparation of manuscripts and emphasizes the importance of conciseness and brevity. Succeeding reports reiterate this need. In the editor's report for 1926, Dr. Stakman stated: "Every author, no matter how experienced he may be, should submit his manuscript to some colleague before submitting it for publication. . . . About 90 per cent of the authors should enlist the aid of a professor of English or an experiment station bulletin editor." This advice is as pertinent in 1954 as it was in 1926.

Over the years, it has become apparent that no amount of advice in editors' reports will do much to improve the quality of manuscripts. The most effective method for bringing about the desired improvement seems to be the rejection of those manuscripts that do not measure up to a prescribed standard. This policy has been in effect for a number of years and the prescribed standard has been gradually raised. Further improvement along these lines is anticipated.

Last year, Council authorized preparation and publication of a paper to acquaint members of the Society with present editorial practices and policies. The writing of this paper is virtually complete; the paper should appear in print in a few months. If it is generally read, authors should more easily prepare manuscripts in the style required and both authors and editors will save much time and effort.

The largest single volume of PHYTOPATHOLOGY, Volume 24, published in 1934, contains 1386 pages, which is roughly equivalent to 730 pages of the present format. The 163 technical articles and notes in Volume 24 average 4.2 pages when adjusted to the present double-column format. In comparison, Volume 43, with 702 pages, contains 183 technical articles and notes, which average 3.5 pages. With the passage of years, articles in PHYTOPATHOLOGY have become briefer; whether they have become more concise cannot be easily ascertained.

The number of articles increased from 110 a volume in 1925-1934 to 145 a volume in 1935-44 to 148 a volume in 1945-54, an increase of 59 per cent from 1925 to 1954. During the same period, the membership of the Society increased from 680 to 1620, or 138 per cent. Thus it appears that interest in the Society, and in the science of plant pathology, is increasing at a faster rate than the output of research in the field. Or does this indicate that PHYTOPATHOLOGY is attracting a smaller proportion of workers' output in 1954 than it did in 1925? Or does it mean that it is more difficult to make a contribution to plant pathology in 1954 than in 1925? The true measure of success in science is not the volume of contributions but the quality. If it may be assumed that the average quality was as good 19 years ago as today, then the number of articles in PHYTOPATHOLOGY should be increased by about 50 per cent if the science is to progress as rapidly as the interest in it increases.

From October 1953 through September 1954, PHYTOPATHOLOGY published 720 pages of technical material, 6½ pages of News, 103½ pages of advertising matter, and a 28 page index and list of errata. Included in the technical material were 128 articles, 15 symposium papers, 28 Phytopathological Notes, 3 obituaries, and 11 book listings; 2 notes for the Interdepartmental Committee on Pest Control; the Report of the 45th Annual Meeting of the Society; 206 abstracts for the 46th Annual Meeting of the Society; the Report and 38 abstracts for the 35th Annual Meeting of the Pacific Division; the Report and 20 abstracts for the 45th Annual Meeting of the Northeastern Division; the Report and 20 abstracts for the Annual Meeting of the Southern Division; and the Report and 15 abstracts for the Annual Meeting of the Potomac Division.

On July 27, 11 manuscripts were in the hands of reviewers, 7 in process of editing after being returned from reviewers, 19 returned to authors for revision, 9 in press, and 26 in galley proof. During the year, 29 manuscripts were rejected by the editors or withdrawn by authors.

Once again, it is my privilege to thank the Rockefeller Institute for Medical Research and the Sustaining Associates for financial assistance and to express appreciation to Mr. John Ferguson, 3rd, and Staff of Monumental Printing Company for their excellent service, to the Editors and Associate Editors for much good advice, to the Business Manager and other officers of the Society for their cooperation, to Mr. and Mrs. Wilson Borland and Mrs. Betty Ann George for editorial assistance, and to Mrs. Carrol E. Cox and Dr. W. F. Jeffers for indexing volumes 43 and 44.

W. C. PRICE, *Editor-in-Chief.*

Report of the Editor of News. During 1954 a half page of news has appeared in each number of PHYTOPATHOLOGY with the exception of the May issue when a full page was devoted to information concerning the 1954 annual meeting. Through the cooperation and assistance of the Committee on Local Arrangements and the Estes Park Conference Camp, a photograph of the entrance to the camp was used as an illustration for the upper half of the news page. If it seems desirable, a similar arrangement is contemplated for future meetings. Expansion of the news section to a full page in each issue has been considered periodically. At present, the burden of regular work prevents the editor of news from taking on the additional load involved. Also the number of news items, both solicited and voluntarily contributed, is not sufficient to warrant doubling the space at this time.

K. W. KRIETLOW, *Editor.*

Report of the Committee on Donations and Legacies. This Committee recommends:

1) That the present list of 10 recipients of gratuitous subscriptions to Phytopathology be continued for the year 1955.

2) That a change be made in the procedure for selecting persons or institutions to receive gratuitous subscriptions so that such selection will henceforth carry no implication of financial distress of the recipient nor of charity on the part of this Society, but will be made as a reward for distinguished service to phytopathology. To this end, this committee will make recommendations, when appropriate occasion exists, of persons or institutions which it deems worthy, on an *honorary* basis, of receiving a complimentary subscription to Phytopathology. The Council may then, in its discretion, accept or reject the committee's recommendation. It should perhaps be a corollary of this recommendation that the complimentary subscription will continue thereafter without further action by the recipient during his lifetime.

3) That the status of this committee be reduced to that of a subcommittee of the Standing Committee on Finances and Investments, so that its actions may be closely correlated with the policies of the latter committee through its chairman.

We especially urge the Council and the Committee on Finances and Investments to give consideration to previous recommendations of our committee to set up definite goals and purposes for the accumulation and utilization of the Society's reserve funds. The lack of definitely projected needs and uses for additional endowed funds is a continuing hindrance to any effective campaign for obtaining funds by donations and legacies.

FREEMAN A. WEISS, *Chairman.*

Report of the Committee on Finances and Investments. Total invested funds: $59,008.78.

Common stock holdings, originally costing $10,999.75 returned $581.42 during the past fiscal year, a yield of 5.3 per cent. As of closing, July 13, 1954, our stock holdings were worth $12,938.00. Our only stock transaction during the year was the purchase of 3 shares of A.T.&T. for $435.34, an average cost per share of $145.11.

Our investments in Federally insured savings and loan associations total $45,658.80. These accounts accrued $1,378.69 during the year, a yield of 3.0 per cent. New savings and loan accounts opened this past year were $6,000 with Decatur Federal, and $4,000 with Fidelity Federal. Both new accounts earned 3.5 per cent.

Miscellaneous investments of $2,350.23 include U. S. Savings Bonds, 7 shares of Family Finance Corporation, and an account at the New Haven Savings Bank.

Our total invested funds earned 3.4 per cent.

Further details are in the Chairman's hands and will be furnished on request.

R. H. WELLMAN, *Chairman.*

Report of the Committee on International Cooperation. The Committee has continued to promote, indirectly in some cases, certain concrete projects of international importance, especially 1) encouragement of the Plant Protection Bulletin, and 2) certain regional cooperative activities in connection with epidemic diseases of regional importance.

On September 30, 1953, 5 pathologists, 4 entomologists, and 2 agronomists met with Dr. Lee Ling in Mexico City and discussed ways by which the Plant Protection Bulletin could be encouraged and supported. Dr. Ling stated that the size of the Bulletin can be increased to handle a greater volume of notes and papers. It is suggested, therefore, that further effort be concentrated during the ensuing year to help Dr. Ling and his staff to make the publication as useful as possible, as volunteer effort is necessary. Attempts will therefore be made to arouse still greater interest during the coming year and to systematize the effort more effectively.

Certain members of the Committee have helped encourage the Uniform Rust Nurseries in Latin America, which are not a Committee activity but which are carried on cooperatively by the U. S. Department of Agriculture and certain other agencies. It is hoped to encourage this work still further and to try to promote certain regional experiments on late blight of potatoes.

The question of closer international cooperation in the western hemisphere on such epidemic diseases as the cereal rusts, late blight of potatoes, and others will be discussed at the forthcoming Third Latin-American Congress of Plant Geneticists, Plant Pathologists, Entomologists, and Soil Scientists, to be held in Colombia in June, 1955. The chairman of the APS Committee on International Cooperation is a member of the organizing committee of the Latin-American Congress, and the members of the APS will be consulted regarding proposals to be made.

In addition to the specific activities mentioned above, various members of the Committee have been actively promoting international or regional cooperation in other parts of the world on epidemic diseases, particularly cereal rusts. The chairman and at least one other member of the Committee, Dr. K. Starr Chester, plan to attend the Jubilee meeting of the Association of Applied Biologists (British) in September, 1954, and will consult with respect to any joint activities that may be initiated in consultation with British and other plant pathologists attending the meeting.

E. C. STAKMAN, *Chairman.*

Report of the Placement Committee. During 1954, 46 plant pathologists have had their applications in the active files of the Placement Committee. Requests from prospective employers for applicants to fill 20 positions have been received. Eighty-one applications have been sent out to 20 employers.

Incomplete records show that at least 2 plant pathologists have obtained positions during 1954 through the efforts of the Committee.

EDWIN E. HONEY, *Chairman.*

Report of the Committee on Public Relations. After considerable searching and screening for a man who had both time and ability to publicize some of our plant disease research papers, Robert Rathbone was selected. Since his appointment in March, he has prepared 4 excellent articles which have been published. At present he is working on a plant pathology career brochure which we expect to be published by the Office of Information, U. S. Department of Agriculture. Press releases covering about 20 papers which appeared to have public appeal will be issued at the time of their presentation at Estes Park.

We feel that if more of our Society members would assume the responsibility for sending copies of their papers to the Public Relations Committee at the time they are accepted for publication, the effectiveness of the Committee's activity would be greatly enhanced. In other words, we now have the vehicle for good public relations work and what we need is more source material.

PAUL R. MILLER, *Chairman.*

Report of the Committee on Plant Disease Prevention. The Committee did not meet during the past year. We are following closely developments along 3 lines: plans for establishing a system of indexing imported fruit plants for virus diseases, the increased concern in the mid-west over the need for a more widespread recognition of buckthorn as a noxious weed, and the growing international concern over the possibility of virulent plant pathogens spreading from Pacific Islands which were formerly much more isolated than at present.

E. B. LAMBERT, *Chairman.*

Report of the Committee on Regulatory Work and Foreign Plant Diseases. The Committee held 2 meetings;

the first at Washington, D. C. and the second at the Plant Introduction Gardens of the United States Department of Agriculture, Glenn Dale, Maryland.

The reports (as printed in PHYTOPATHOLOGY) of this Committee from 1927 to the present time were assembled to 1) facilitate a review of the previous Committee activities and recommendations and 2) serve as a guide for future deliberations.

The Director of the Crops Regulatory Program of the Federal Department of Agriculture (formerly the Bureau of Entomology and Plant Quarantine) submitted to the Committee for its consideration a series of 11 topics. Committee considerations were restricted to 7 of these.

This Committee has, in previous reports, recommended the regulation of the importation of cultures of plant pathogens. Attempted Federal legislation on this subject has not been attained; various technicalities ascribed as the reason therefor. A new draft for an amendment of the Federal Plant Quarantine Act of 1912 has been prepared and is now being circulated among the various concerned Federal Agencies for examination. This proposed legislation provides for the establishments of safe-guards against snails, soil, insects, nematodes, viruses, and cultures of living micro-organisms. The Committee reaffirms its approval of these efforts and recommends that the Society take appropriate action endorsing such legislation in principle and transmitting a copy of its action to the Secretary of the United States Department of Agriculture.

The Committee recommends the discouragement of requests for the importation of cultures of plant pathogens because of the risk of the introduction of races with a host and varietal range which could lead to unfortunate consequences. Immediate study should be given to this problem so that concerned plant breeders may, without undue delay, adjust their researches to a national policy on this subject.

The Committee recommends to the Director of the Federal Crops Regulatory Programs the initiation of studies of the practicability and usefulness of indexing imported plant material of selected genera of plants susceptible to virus infections.

Committee deliberations on the remainder of the topics accepted for consideration have not progressed to the stage which permits definite, crystallized recommendations. The Glenn Dale Meeting was devoted largely to the acquisition of information pertaining to the problems of Postentry Quarantine.

E. G. REX, *Chairman.*

Report of the Committee on Seed and Plant Material Certification. The program of this committee has been described in a progress report published in the Plant Disease Reporter 38: 521-528, 1954. The outstanding activities during this past year include: 1) establishment of good liason with the International Crop Improvement Association 2) organization of subcommittees and preliminary study of the problems in related groups of plants, and 3) solicitation of plant pathologists through publication of our progress report explaining our program, objectives, subcommittee organization with a new request for participation. The committee is now well organized for handling these problems, and it is hoped that more general participation by plant pathologists will provide the information necessary to accomplish our objectives. The committee plans to circulate descriptions of pressing problems, reports of meetings with the I.C.I.A., summaries of contributions, and preliminary proposals for new or revised pathological standards by periodic publication in suitable journals. It wishes to express appreciation for the support and cooperation from the International Crop Improvement Association and to Dr. Paul R. Miller for assistance in preparing our material for publication in the Plant Disease Reporter.

JOHN R. HARDISON, *Chairman.*

Report of the Committee on Resolutions. *Whereas,* this, the 46th annual meeting of the American Phytopathological Society held at Estes Park, Colorado, has been one of the most pleasant and profitable of our annual conventions, and,

Whereas, this, the 46th year of the official existence of our society has been so highly successful, and with other evidence of good leadership, and,

Whereas, such performance should not go unrecognized,

Be It Therefore Resolved, that we express our sincere appreciation to the following for their unselfish contributions to the aforementioned successes, to wit:

1) To the Pacific Division of the American Phytopathological Society and The Potato Association of America who by their participation in a joint session contributed so greatly.

2) To certain local Colorado Society members for conceiving the idea of and extending the invitation to meet at this delightful place on a family basis and thereby setting the precedent for similar future meetings, at discreet intervals, considerably west of the Mississippi.

3) To W. J. Henderson, chairman, and to the rest of the committee on arrangements for their efficient and extensive efforts in attending to the vast multiplicity of details prerequisite to the success of such a convention as this.

4) To W. G. Ruesch, manager of the YMCA Conference Camp and to his staff for their courtesies and efficient attention to our needs for housing, food, recreation, and entertainment. Especial recognition is due the staff of the dining hall for the quantity and quality of the food so promptly and well served the multitude of hungry souls that poured into said hall as soon as they could get in, and to the variety and continuity of recreation afforded our wives and children.

5) To Shell Chemical Corporation, Rohm and Haas Chemical Company, E. I. du Pont de Nemours and Company, United States Rubber Company, and the Naugatuck Chemical Division for their financial contributions toward the success of the annual banquet and entertainment.

6) To the Wyoming delegation for their most unique banquet entertainment.

7) To the Program Committee and to the sub-committees of the various symposia for organization of the program and to the several section chairmen for their efficient services in maintaining proper schedule in the presentation of papers.

8) To the students of Colorado A. and M. College who operated the projection equipment.

9) To our officers, retiring president George F. Weber, Secretary Glenn S. Pound, Treasurer and Business Manager Saul Rich, and our retiring Editor-in-Chief W. C. Price for their generous and productive expenditure of time and energy toward the duties of their respective offices; to the rest of the Council for efficient attention to the affairs of the Society; to the several standing and special committees, and the various society representatives, for efficient dispatch of their responsibilities.

GEORGE W. FISCHER
BYRON R. HOUSTON

Elections and Appointments. A committee was appointed by the Council to count the ballots for the election of new officers. The following were declared elected: President Elect, Helen Hart; Vice President, George Fischer; Councilor-at-Large, W. C. Snyder.

Upon recommendation of the Council, the Society approved the appointment of A. Frank Ross as Editor-in-Chief of PHYTOPATHOLOGY for a 3-year term beginning January 1, 1955; of Ralph M. Caldwell as Editor and of Earle W. Hanson, F. P. McWhorter, R. P. True, and V. W. Cochrane as Associate Editors of PHYTOPATHOLOGY, all for 3-year terms beginning January 1, 1955.

Representatives and Committees as listed above were appointed and approved.

The Society approved recommendations of the Council as follows:

1) That $1,000.00 be set aside to be drawn on by the Business Manager of PHYTOPATHOLOGICAL CLASSICS to reprint Phytopathological Classics 1 and 2 with the instruction that the Committee on Phytopathological Classics follow the policy of reprinting classics so that copies of all classics will be available at all times.

2) That the Representative to the American Institute of Biological Sciences be authorized, with the approval of the Council, to apply to AIBS, after its next annual meeting, for a change in Society status from that of a Member Society to that of an Affiliate Society.

3) That the International Microbiological Congress be petitioned to elevate the "Plant Virus Subsection of Virus Nomenclature" of the "Virus Nomenclature Subcommittee" of the "International Committee on Bacteriological Nomenclature" to Subcommittee status and that the Chairman of the Plant Virus Subsection of Virus Nomenclature be made a full member of the International Committee on Bacteriological Nomenclature.

4) That the fund appropriated to the Public Relations Committee for 1954 of which some $400 has been spent be restored to $1,500 for similar public relations writing in 1955.

5) That $500 be appropriated for publication of a career brochure in plant pathology when prepared by the Committee on Teaching of Plant Pathology and when approved by the Council and by the Editorial Board of PHYTOPATHOLOGY.

6) That item D of Standing Rule 6 of the Constitution which reads

> "The abstracts shall be subject to final acceptance and editing by a reviewing committee appointed by the Editor-in-Chief. Accepted abstracts will be published in PHYTOPATHOLOGY at the expense of the Society, provided that not more than 2 abstracts under the sole or joint authorship of one member, that are presented at the meetings of the Society or the Divisions, shall be published at the Society's expense within one calendar year."

be changed to read as follows:

> "The abstracts shall be subject to final acceptance and editing by a review committee appointed by the Editor-in-Chief. Accepted abstracts will be published in PHYTOPATHOLOGY at the expense of the Society."

and that item C of Standing Rule 7 which reads

> "*Publication.* Summaries of the proceedings of the annual meetings of Divisions shall be published in PHYTOPATHOLOGY at the Society's expense. Abstracts of papers presented at Division meetings also may be published in PHYTOPATHOLOGY subject to the rules governing the preparation and publication of abstracts as stated in Rule 6, and with the specific limit of 2 on the number of abstracts that any member may have published at the Society's expense within 1 calendar year."

be changed to read as follows:

> "*Publication:* Summaries of proceedings of the annual meetings of Divisions shall be published in PHYTOPATHOLOGY at the Society's expense. Abstracts of papers presented at Divisional meetings may also be published in PHYTOPATHOLOGY, subject to the rules governing preparation and publication of abstracts as stated in Rule 6, except that no more than 2 abstracts presented by any one member (as sole or joint author) at the Annual Meeting of the Society and the corresponding Annual Meeting of the Divisions shall be published at the Society's expense."

7) That the second sentence of item C of Standing Rule 6 which says,

> "The text must not exceed 200 words in length."

be changed to read

> "The text must not exceed 1300 typewriter spaces in length."

8) That the Society endorse and approve the Resolution dealing with the Importation of Fruit Plant Materials From Foreign Countries For Research Purposes as submitted by the Interregional Technical Committee for Stone Fruit Virus Research, a copy of which is given below.

The importation of Fruit Plant Materials from Foreign Countries for Research Purposes. "For purposes of research on virus diseases of fruit plants, there is a great need for the importation from foreign countries various species of plants not occurring in the United States. Use of such material for testing for commercial value, in breeding work for production of new varieties, for test plants in host range studies of known virus diseases, or for indexing plants suspected of carrying virus may provide valuable research tools not obtainable if only domestic species are used.

"The United States Federal Nursery Stock, Plant, and Seed Quarantine No. 37, revised effective December 5, 1950, prohibits the importation of certain plant materials, in some cases entirely and in other cases only from certain countries. This last provision is based on the known occurrence of certain diseases in the areas of origin.

"Under (b) of Section 319.37, provision is made for importation of otherwise prohibited plant material by the U. S. Department of Agriculture for experimental or scientific purposes. The procedure for such introduction has necessarily had to be based on practicality and has consisted of propagation and retention of the material at the quarantine station at Glenn Dale, Maryland, while visual observations were made for pests. This procedure has been slow and does not provide protection from viruses which may be carried in the material in a latent state, but which may be destructive to other varieties or species.

"*Whereas,* this procedure is considered now inadequate for protection from viruses in plant materials, it is suggested that modifications be made to give greater safeguards and more expeditious handling.

"For tree fruits, it is well known that indexing procedure is necessary for detection of many viruses and is better by far than is visual inspection. Therefore, it is suggested that index procedures be instituted for all fruit plant materials being introduced and propagated at the Glenn Dale isolation station or any substations thereof before they are placed in use. For example, for stone fruit materials, if the plants are indexed on appropriate hosts, as listed by the Interregional Subcommittee on 'Host Range Studies on Virus Diseases of Stone Fruits,' it would be considered that adequate precautions will have been taken."

9) Accepted into annual and student membership, 71 and 54 applicants, respectively.

Announcements of Meetings. The Council announced that the 1955 meeting would be held in Atlanta, Georgia, December 28-30 in conjunction with the American Association for the Advancement of Science.

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Page 565, column 1, line 23 *read* to 2 of 4 *for* of 2 of 4

Page 591, column 2, line 7: *read* 1897 *for* 1894

Page 602, column 1, paragraph 1, line 4: *add* This reagent is dithizone extracted.

Page 604, column 1, line 3 from bottom: *read* established stands *for* establishing stands

Page 614, Table 2, heading of column 3: *read* Days on plant *for* Days of plant

Page 650, column 1, line 2: *read* core[4] *for* core[5]

Page 659, column 1, line 20: *read* benzene for benzol; *read* tosyl *for* toluol

line 21: *read* tribromo *for* tribrom

line 24: *read* tosyl *for* toluol

line 25: *read* tosyl *for* tusuol

line 30: *read* tosyl *for* toluol

line 36: *read* benzene *for* benzol

line 37: *read* benzene *for* benzol

Page 660, column 1, line 53: *read* sulfanilamidoisoazole *for* sulfanilamid-isoazole

column 2, line 13: *read* benzene *for* benzine

Page 661, Table 2: *read* Ammate *for* Amate

Page 662, column 1, line 27: *read* benzene *for* benzine

line 36: *read* zerlate *for* zeorlate

line 38: *read* triethanol *for* trienthenol

line 39: *read* paradichlorobenzene *for* paradichlorobenzine

line 41: *read* lactate for lacate; *read* Ammate for Amate

column 2, line 4: *read* tocopherol *for* trocopherol

line 8: *read* palmitate *for* palmetate

line 12: *read* phenoxy *for* phenozy

line 19: *read* gramicidin *for* gramosidin; *read* tyrocidine *for* tyrosidin

line 30: *read* sulfanilamidoisoazole *for* sulfanilamido-isolazole

Page 676, column 2, line 42: *read* Fig. 2 *for* Fig. 1

Page 678, column 1, line 13: *read* Fig. 3 *for* Fig. 2; line 37: *read* Fig. 2 *for* Fig. 1.

column 2, line 47: *read* Fig. 4 *for* Fig. 3

Page 679, Table 2, footnote g: *read* Fig. 4 *for* Fig. 3

column 2, line 25: *read* Fig. 5 *for* Fig. 4

Page 680, column 1, line 12: *read* Fig. 5 *for* Fig. 3

Page 689, Fig. 2 legend: grazing angle *read* 21° *for* 11°; magnification *read* ×60000 *for* ×27500

Page 690, column 1, Fig. 3 legend: *add* after "particles" . . . by two different methods, a) shadow measurement and b) measurement vertical to the shadow (see Table 4).

ERRATA, VOLUME 44

Page 41, column 1, line 24: *read* 17 *for* 7

Page 60, column 2, Literature citation 5: *read* S. D. Garrett *for* S. S. Garrett

Page 64, column 2, Figure 1 is inverted

Page 118, column 2, line 6: *read* effected *for* affected

Page 144, column 1, line 4: *read* cultures of *for* cultures on

Page 194, column 1, Table 1: H 3 and H 8 *should be in* Low (L) section *instead of* Moderate (M) section

Page 232, Figure 2 is inverted

Page 269, column 2, sentence 1: *read* $L = 1556 \ln (3+2p-p^2) + 457 \ln (1-2p+p^2) + 196 \ln (1+2p-p^2)$. *for* $L = 1556-(3+2p-p^2) + 457-(1-2p+p^2) + 196-(1+2p-p^2)$.

Page 285, column 1, Figure 1 legend: *read* Y = .1 + .392X *for* Y.1 .392X

Page 297, column 1, Literature citation 3: *read* Studier *for* Studies

Page 303, column 2, line 17: *read* Cucurbita pepo L. *for* Cucurbita maxima Duchesne

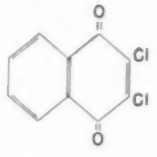
Page 309, column 2, line 9 from bottom: *read* Cucurbita pepo L. *for* Cucurbita maxima Duchesne; line 6 from bottom: Dickinson is var. of Cucurbita moschata Duchesne

Page 319, column 1: transpose lines 2 and 3

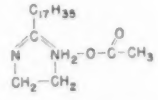
Page 324, column 1, Literature citation 5: *read* Fenne *for* Fenen

Page 370, column 1, line 49; and column 2, line 38: *read* 2-heptadecyl-2-imidazoline acetate *for* 2-heptadecyl glyoxalidine acetate.

Page 370, column 2: *for* the structural formula for dichlone (2,3-dichloro-1,4-napthoquinone) *substitute the following*:



Page 370, column 2: *for* the structural formula for glyodin (2-heptadecyl-2-imidazoline acetate) *substitute the following*:



Page 394, column 1, line 5 from bottom: *read* H 309 *for* Celaya

Page 417, column 2, line 41: after São Paulo[3] *add* from Bolivia

Page 482, column 1, line 37: *read* Bayol F *for* Mayol F

Page 484, column 1, line 24 from bottom: *read* Cardona A, Canuto *for* Canuto, Cardona A.

What Plant Food Means To The Grower

Plant food, properly used, is one of the cheapest aids to success a grower can employ. It will save him labor by growing his crops on fewer acres; increase yields by thickening and strengthening the stand of crops and making them more disease-resistant; improve the quality to obtain top prices; and prevent loss of his soil assets by preventing erosion and maintaining fertility. In order to produce as efficiently as possible, the grower is turning more and more to the agricultural scientist for the results of research and practical information on the use of plant food.

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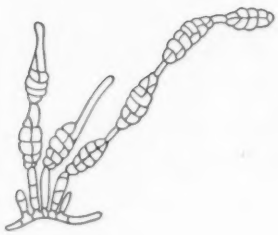

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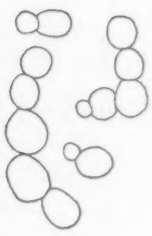

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With this issue, W. C. Price completes his term as Editor-in-Chief of PHYTOPATHOLOGY. The Society is indebted to Dr. Price for guiding our Journal during the difficult period of changing to a new format. The standard he has set for PHYTOPATHOLOGY makes our Journal outstanding among scientific publications. That this standard of excellence will be maintained is assured as we welcome our new Editor-in-Chief, A. Frank Ross. Dr. Ross has served the Society in several capacities and is well qualified to accept the responsibilities of the Editor's chair. All manuscripts for publication in PHYTOPATHOLOGY should henceforth be addressed to Dr. A. F. Ross at the Department of Plant Pathology, Cornell University, Ithaca, New York.

The Gold Medallion for Distinguished Service to New Jersey Agriculture was awarded recently to Life Member, Dean and Director, William H. Martin of the New Jersey College of Agriculture and Experiment Station. The medallion was presented by the New Jersey Agricultural Society which was founded in 1781.

New Head of the Department of Botany and Plant Pathology, Utah State Agricultural College is Ralph W. Ames. Dr. Ames succeeds S. W. Boyles who has been Acting Head since the death of F. B. Wann.

Congratulations to phytopathologist, J. R. Shay of Purdue University who was recently awarded the Joseph Harvey Gourley award in pomology by the American Society of Horticultural Science.

Phytopathologist, T. W. Bretz, formerly forest pathologist in the U. S. Department of Agriculture has been appointed Professor of Forestry (Forest Pathology) on the staff of the University of Missouri and the Missouri Agricultural Experiment Station.

A symposium on Forage Crop Diseases and Grassland Farming will be held by the Crops Section of the American Society of Agronomy and the Southern Division of the American Phytopathological Society at the meetings of the Association of Southern Agricultural Workers, February 7-9, in Louisville, Kentucky.

The Northeastern Division held its tenth annual winter meeting at West Springfield, Massachusetts, November 4-5, with well over 100 people in attendance. In addition to three general sessions in which 26 papers were presented, an evening program consisting of informal papers on field comparisons of fungicides for the control of fruit and vegetable diseases was arranged by Dr. D. H. Palmiter. Officers elected for 1955 are: A. B. Burrell, President; A. C. Tarjan, Vice-President; B. H. Davis, Secretary-Treasurer; and E. G. Rex, Councilor.

Deaths

Vera K. Charles, Charter and Life Member of the Society, died November 2. Miss Charles conducted investigations in the Division of Mycology, Bureau of Plant Industry for 39 years until her retirement in 1942. Her chief interests included edible and poisonous mushrooms, entomogenous fungi, and fungi pathogenic to man and animals.

L. R. Tehon, Head, Section of Applied Botany and Plant Pathology, Illinois State Natural History Survey, Professor of Plant Pathology, Graduate College, University of Illinois, died October 17 at the age of 59. Dr. Tehon conducted extensive surveys on the flora of Illinois, and investigated tree and floricultural diseases.

Editor: K. W. KREITLOW, Plant Industry Station, Beltsville, Md.

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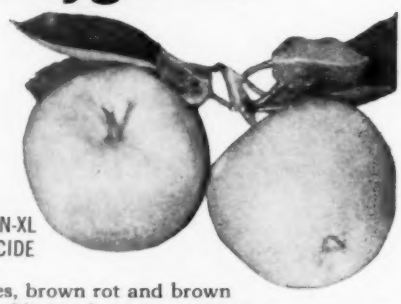

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TOBACCO varieties developed by Federal and State Agricultural Experiment Stations and commercial tobacco breeders have resulted in many benefits for the Flue-cured grower. However, new varieties, with their specialized characteristics, have added to the grower's responsibility. He must learn the characteristics of the varieties available in order to make a sound choice for his particular conditions. Practical help in choosing suitable varieties can be obtained from Federal and State Agricultural Experiment Stations.

Careful thought in selecting the right variety will reward the Flue-cured grower with crops of higher quality.

Put Quality *First*

In Choosing Flue-Cured Varieties

igh quality tobacco of average acre-yield ill, in the long run, give a greater return han high yields of poor quality tobacco. here are varieties available such as 402, irginia Gold, Golden Harvest, and Golden re that will produce excellent quality nder good growing conditions, and fair-to-ood quality under more adverse conditions. n the other hand, certain varieties such as icks, White Gold, and Bottom Special will roduce good quality under favorable condi-ons, but will fall short if adverse weather onditions occur.

DISEASE RESISTANCE

nowledge of the tobacco diseases present in n area gives the grower further guidance n choosing varieties. The successful use of isease-control measures, for example disease-esistant varieties, is based first of all upon he correct identification of the disease, and ost farmers realize that they cannot afford o take a chance on guesswork or uncertain iagnosis. Many of the disease-resistant varie-es of tobacco that have been developed are qual to non-resistant varieties in both yield nd quality. Certain of the newer disease-re-istant varieties are more resistant to drought.

A common pitfall is the idea that the use f disease-resistant varieties makes rotation ractices unnecessary. Besides the fact that ontinuous tobacco culture on the same land nds to lower quality, the use of disease-esistant varieties without rotation diminishes he effectiveness of the resistant factor. A ood rotation system not only improves the uality of Flue-cured tobacco, but builds up he land and reduces the danger of disease.

YIELD

Some varieties respond more favorably to heavier fertilization from a yield and quality standpoint than do others. This differential response should be strongly considered when selecting a variety of tobacco. In general, the broader leaf types are the higher yielders. Closely associated with the yield are such characteristics as high leaf number and lateness. Once tobacco flowers, no more leaves are added on the primary stalk. These characteristics are being strongly considered in the present breeding programs.

CHEMICAL QUALITIES

Some tobacco varieties produce lower nicotine-content than others. The varieties that are low nicotine-producers are usually high sugar-producers. A moderately-high level of sugar is necessary for mildness, although too much sugar may be associated with slick texture and lack of flavor. Varieties differ also in their ability to absorb the mineral nutrients from the soil. The uptake of mineral constituents, especially potash, has a bearing on the grain and burning quality, hence the smoking quality, of the leaf.

HANDLING QUALITIES

A variety that has the ease of curing of Hicks is worth much to the farmer. Breeders are continually working to incorporate the ease-of-curing characteristic into new and better varieties. Also, they are striving to produce varieties that are not brittle.

SOIL

Since some varieties of tobacco are much better adapted to certain soils than to others, the grower should study the results of an accurate analysis of his soil. Tobacco, more than many crops, requires well balanced soil fertility to produce a profitable yield of good quality tobacco. If, for example, the soil were found to contain too much organic material —which may make the plant's content of nicotine and other forms of nitrogen too high as a result of the excess nitrogen in the soil —one of the lower-nicotine varieties might be considered to counteract this surplus to some extent. A soil test, by indicating the fertility, helps the grower to choose a variety suitable to that level. A thorough knowledge of the soils on the farm is important.

CLIMATE

Some varieties of tobacco are well suited to areas that have a heavy rainfall, while others withstand drier areas. Seeding, planting, and harvesting dates are influenced by the climate, and these factors should be considered in choosing varieties.

For more information on choosing a variety for your particular conditions, get in touch with your County Agent, other Extension Workers, or Vocational Agriculture Teachers.

The American Tobacco Company

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Flue-cured advertisement appearing in farm publications. It is representative of the series started in the Burley area and later extended to the Flue-cured area.

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